THE NUTRIENT REQUIREMENTS OF WHITE CLOVER ON HILL SOILS

by

Anne Rangeley

Thesis presented for the degree of Doctor of Philosophy of the University of Edinburgh in the Faculty of Science.

1980

DECLARATION

I declare that the composition of this thesis, and the investigations described herein, are my own work, except where reference has been made to published literature.

C O N T E N T S

ABSTRACT

THE NUTRIENT REQUIREMENTS OF WHITE CLOVER ON HILL SOILS

This investigation was part of a larger study to understand the main factors which affect the establishment, growth and persistence of white clover (T. repens L.) in improved hill pastures. Precise knowledge of the nutrient requirements for both the establishment and maintenance of white clover on hill soils is needed to make pasture improvement more reliable and less costly. The specific aims of the study were:

1. To identify the magnitude of response of white clover to the major nutrients and lime when grown on acid hill soils.
2. To establish critical levels of nutrients in the shoot.
3. To evaluate possible benefits to the phosphorus nutrition of the plant from inoculation with mycorrhizal fungi.

Experiments were carried out in glasshouse, growth room and field, using soils representative of the main groups found in hill areas, although the emphasis was on deep peat. In laboratory experiments, white clover after inoculation with Rhizobium was not found to respond to nitrogen nor did this element interact with phosphorus or potassium. However, the plants responded markedly to phosphorus and potassium; the response to one element was greatly influenced by the level of application of the other.

There was a positive response to lime up to a pH of 5.5; thereafter, further lime reduced growth, apparently through its effect on the phosphorus nutrition of the plant. There was little response to magnesium. Field experiments with an established pasture on deep peat soil confirmed that lack of phosphorus and potassium can severely limit growth of white clover. The value of results obtained from laboratory experiments for prediction of response to nutrients in the field is discussed.

Critical concentrations of phosphorus, potassium and magnesium in the dry matter of the shoots were 0.20% P, 0.9% K and 0.29% Mg. Although the critical concentration of calcium was not determined precisely, the data suggest that a concentration of 1% or less in whole shoots restricts growth. The use of shoot analysis and critical concentrations to determine the need for maintenance dressings of fertilizer is considered.

Mycorrhizal fungi were successfully introduced into the roots of white clover in both laboratory and field experiments; the responses to inoculation depended on soil type, introduced endophyte, the presence of indigenous endophytes and the environmental conditions. In laboratory experiments with a deep peat soil there were marked responses in dry matter production and nutrient uptake, coupled with beneficial effects on nodulation and nitrogen fixation. Low temperatures and the wetness of this soil

were probably the major environmental factors which prevented growth responses in the field.

With two brown earth soils in the laboratory there were no responses in dry matter production, possibly because the soils contained a high density of indigenous endophytes. However, Glomus caledonius did significantly increase yield in the field on one of the soils during the second year of growth. By contrast, on the other brown earth soil, Glomus mosseae (L1) significantly depressed yield in the year of sowing. It is concluded that mycorrhizas should be collected from well established white clover pastures throughout Britain and screened in the laboratory, care being taken to match the mycorrhizal endophytes with both cultivar of white clover and strain of Rhizobium.

The study has laid the basis for further nutrient work with other hill soil types and with mixed swards of white clover and companion grasses in the field. It has also shown that, although mycorrhizas have varying effects on clover growth in the field, some can be very beneficial and further investigation is justified.

ACKNOWLEDGEMENTS

I am very grateful to Professor J.M.M. Cunningham and the Agricultural Research Council for allowing me to use the facilities at the Hill Farming Research Institute to do the study. I am particularly grateful to Dr. Peter Newbould, my supervisor at HFRO, for advice throughout the project and for patiently checking and helpfully commenting on the drafts, and to Dr. Ron Thow (Edinburgh University School of Agriculture), my second supervisor, for his help and encouragement.

I am indebted to Dr. Melvin J. Daft (University of Dundee) for introducing me to the mycorrhizas and for valuable discussion and to Dr. Mike J.S. Floate for discussion and advice.

I wish to thank Mr. Colin Evans, John McKenzie and Sandy Ironside for chemical analysis; Glyn Davies for help with statistical analysis; Dr. John Rogers for helping me with the statistical analysis on the computer. I also wish to thank Julia Leask and Mary Thornton who bore with me for several months.

I am indebted to Ian Pitkethly for the excellent diagrams and photographs and to Janet Hall, Liz Reid and Elaine Campbell for the draft typing. My gratitude also goes to Helen Tulloch and Liz Reid for typing the final draft to meet my deadline, and to Elwyn Davies who checked the final copy.

Finally, my thanks go to Professor A.H. Bunting whose faith in me kept me going, and to John for his infinite patience.

GENERAL INTRODUCTION

The speedy establishment and continued growth and persistence of white clover is regarded as the key to successful schemes of pasture improvement in hill soils (Munro, 1970; Newbould, 1974/5; Newbould and Haystead, 1978). The introduction of a proportion of improved pasture and its strategic use to supplement and complement rough grazing in newly designed systems of hill sheep farming has been shown to result in higher output and hence greater financial returns for farmers (Eadie *et al.*, 1976). White clover is required both to fix atmospheric nitrogen and to provide high quality forage for grazing animals (Armstrong and Eadie, 1973; Thompson, 1979). Unfortunately it has a reputation for unpredictability in establishment and production (Wright, 1975; Newbould and Haystead, 1978) and great importance is attached to improvements in both of these characteristics. The present investigation was part of a larger collaborative study at the Hill Farming Research Organisation (HFRO) to examine all aspects of the use of white clover in hill and upland agriculture.

White clover (Trifolium repens L.)

Trifolium repens L. is a member of the family Papilionaceae (formerly Leguminoseae) and is a native of the British Isles, commonly found in grassy places. It is distributed throughout Europe to 71^{0}N, north and west Asia and North Africa (Clapham *et al.*, 1962). White clover is tolerant of the British climate but its

occurrence is limited by soil pH, the calcium and phosphorus level in the soil, the absence of rhizobia and competition from grasses (Spedding and Diekmahns, 1972). The factors which limit the distribution can easily be altered by agricultural practices.

Plant breeders have selected varieties suitable for a range of environmental and farming conditions; those recommended as both tolerant of the climatic and soil conditions in the hills and uplands and as suitable for use in grazed pastures are Aberystwyth S100 or S184, New Zealand Grasslands Huia or Kent wild white (NSCA, 1972; SAC, 1979; NIAB, 1978). Aberystwyth S184 and New Zealand Grasslands Huia were the varieties chosen for use in the present study. They are often used together for hill pasture improvement where Huia with medium small leaves is reputed to establish quickly but is not persistent, while S184 with small leaves takes some time to establish but thereafter is very persistent (NSCA, 1972; NIAB, 1978; SAC, 1979).

Atmospheric nitrogen is fixed by the symbiotic bacterium Rhizobium trifolii in nodules on the roots of T. repens. The fixed nitrogen is available to the clover plant itself and is transferred to the associated grasses by the return of dung and urine from the grazing animal and by the decomposition of roots and nodules (Newbould and Haystead, 1978; Haystead and Marriott, 1979a). Applications of nitrogen fertilizer are not an economical alternative source of nitrogen for the

extensive systems of hill farming (Munro, 1969; Newbould, 1974). Newbould and Haystead (1978) from the available published information, calculated that white clover contributes 90-184 kg N/ha/yr to grass/clover swards on hill land which is equivalent to 128-373 kg fertiliser-N/ha/yr.

The numbers of indigenous rhizobia in acid hill soils are low (brown earth) or in some cases are absent altogether (deep peat). Those that are present are often inefficient fixers of nitrogen (Holding and King, 1963). On deep peats Newbould et al. (1980) have demonstrated yield responses in the field by white clover to inoculation with effective strains of Rhizobium in the establishment year. Intermittent and inconsistent effects of inoculation were observed on other soil types. The authors strongly recommended that white clover be inoculated with the appropriate strain of Rhizobium when reseeds are sown on deep peats. Because the cost of inoculation is small compared with the price of seed and the total cost of hill improvement, inoculation of all clover for hill reseeds was advised (Newbould et al., 1980).

Research on the reasons for the unpredictability of response of white clover to inoculation, on different techniques of inoculation and the use of selected strains of Rhizobium is in progress in other projects at HFRO and was not part of the present investigation. However, to avoid lack of effective rhizobia interfering with the results of the present investigation, a suitable

inoculum was applied to all experiments.

Hill soils and vegetation

British hill soils and the problems they give rise to for agricultural production are described by Floate (1967 and 1977) and the types of vegetation found on hill ground by Birse (1968) and King and Nicholson (1964).

There is a fertility gradient of soils from hill top to valley bottom: the soils at the top of the hill are generally less fertile than those near the bottom. As rainfall increases at higher altitudes, soils are increasingly leached, are weathered more slowly and the organic matter in or on top of the soil profile increases. As the content of organic matter increases, the cation exchange capacity of the soil increases but the base saturation decreases through leaching. Soils become increasingly acidic with hydrogen occupying exchange sites in organic soils, and hydrogen and aluminium in mineral soils (Floate, 1977; HFRO, 1979). The pH of the soil therefore is a useful index of the soil nutrient level (HFRO, 1979).

Hill soils fall into four main categories - gleys, brown earths, podzols and peats - although there are many intergrades and they are associated with fairly distinct types of vegetation. The poorly drained gley soils which occur in valley bottoms have a pH range 4.5-6.0 and support Agrostis/Festuca pasture with Carex and Juncus species and, at the lower end of the pH range, Nardus

stricta. The brown earth soils which range from pH 4.5-6.0 are freely drained, occur on the lower slopes and support Agrostis/Festuca grassland. The number of species associated with this grassland increases with pH; T. repens is often found growing in soils above pH 5.3. The podzols and peaty podzols are freely drained, and the peaty gleys are poorly drained: they have a pH of 4.0-4.5, are found at higher altitudes than the brown earths and are associated with Calluna shrub heaths and grass heaths. In the drier areas the grasses, Nardus stricta, Deschampsia flexuosa and Festuca ovina are found, but Molinia caerulea becomes increasingly more common as the soils get wetter. Deep blanket peat is poorly drained and is found above 200 m in the west and 600 m in the east. The pH is 3.5-4.6 and the vegetation type is a Trichophorum-Eriophorum-Calluna bog (HFRO, 1979). A deep peat, a dry peaty podzol and two brown earth soils were used in these experiments to represent the range of hill soils.

The total nutrient content present in hill soils is not much different to the total nutrient content of lowland soils (Reith, 1973; Floate, 1977). However, most of the nutrients are present in the organic matter and their release through mineralisation is slow. Low temperatures and acidity principally restrict mineralisation (Floate, 1970). In some mineral soils acid weathering releases iron and aluminium oxides which fix phosphorus and render it unavailable (HFRO, 1979). The low concentration of nutrients in the labile pool and the

slow rate at which soil nutrients are replenished can drastically restrict plant growth. Nitrogen is particularly slowly mineralised and is the major restraint to productivity of resown grassland (Munro et al., 1973; HFRO, 1979). Because white clover is relied upon to provide biologically fixed nitrogen for growth of improved hill pastures, rather than mineral nitrogen from fertilizers, it is important that the nutrients required to establish it in hill soils are known more precisely.

Trace element deficiencies can often be apparent or are induced after lime is added to raise soil pH (Lucas and Davies, 1961). No attempt has been made to examine the response of white clover to trace elements in these experiments but, to avoid any deficiencies arising, a solution of the chief trace elements known to affect both growth and nitrogen fixation (Andrew, 1977) was added to all experiments.

Nutrient requirements of white clover

The nutrients needed to introduce white clover into hill soils at the start of a hill pasture improvement scheme (establishment) contrasts with those needed to keep white clover growing well over a period of time when the pasture is grazed (maintenance). The work described here is aimed primarily at establishment. The general principles that lime, phosphorus and potassium have to be added, and the range of levels needed for different soils,

are broadly known (WSAC, 1975; ESCA, 1977; NSCA, 1978; ADAS, 1979). However, with the ever increasing costs of the lime and fertilizers and their application, it is most important for the future survival of the low output hill farming sector of British agriculture that the rates of fertilizer and the balance between different chemicals are known more precisely. It is particularly helpful to have knowledge of the biological response curves (dry matter and/or nutrient content) so that increasingly expensive resources can be used to optimum economic advantage (Fig. 1).

There is little precise information on the nutrient requirements of pasture species grown in hill soils in Britain. Present recommendations are probably based on early work in land improvement, e.g. Ogg and Robertson (1934), lowland recommendations, soil analysis and the experience of farmers and advisors. However, the methods of soil analysis for extractable phosphorus used by the advisory services do not satisfactorily predict the amount of phosphorus taken up by ryegrass or white clover (Pimplaskar et al., 1980) and methods of soil analysis for the other major elemements in hill soils are not entirely reliable (Floate et al., 1977). The objective of the present experiments was to examine the responses in dry matter and nutrient uptake of white clover in terms of fertilizer efficiency, plant deficiency and sufficiency and to identify interactions, if any. It was never the

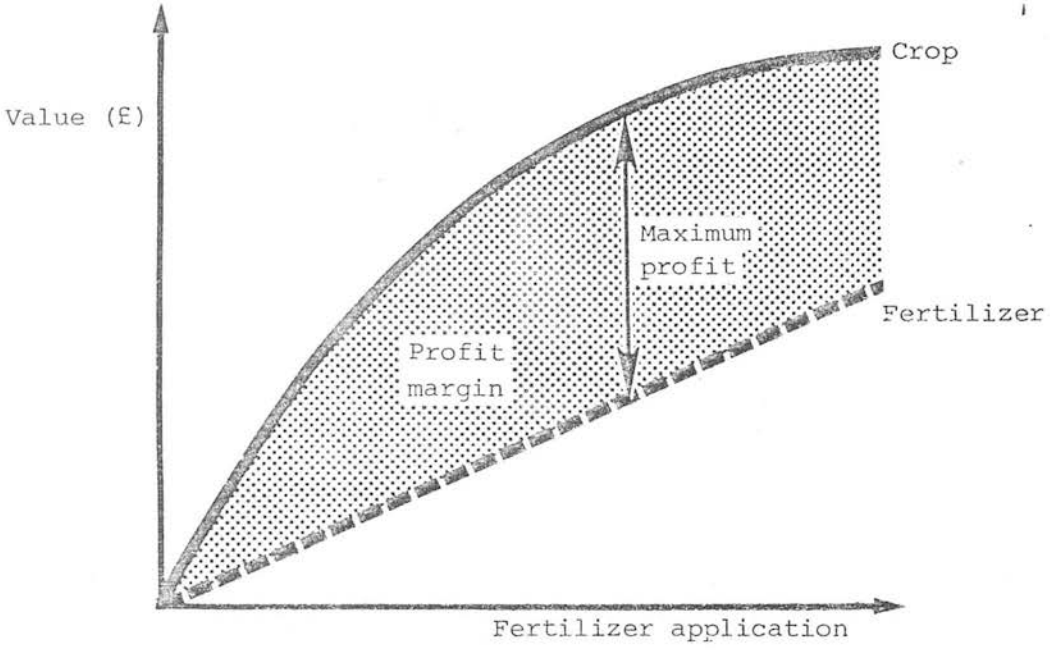


Figure 1. The profit margin from application of fertilizer (after White, 1979)

intention in the experimental work of this thesis to relate assessments of available soil nutrients to growth and nutrient uptake by white clover. However, some of the information adds to the discussion of the relative value of pot and field experiments and to some aspects of soil/plant relationships.

During the course of the study of responses to nutrients it became evident that mycorrhiza might be confounding the results particularly at low levels of added phosphate so work to examine this possibility was also begun.

Thus, the present investigations were undertaken to study the responses in dry matter production and nutrient content of white clover growing in hill soils to additions of the major nutrients (Part I) and the benefits or otherwise of mycorrhiza to the establishment, growth and nutrient content of white clover which follow either an increase in the level of, or a change in the strain of, mycorrhiza which infect the roots (Part II). One of the experiments was aimed at both subjects: the effects of fertilizer treatments are given in Part I (Experiment 2), and the effect of inoculation with mycorrhiza is reported in Part II (Experiment 9).

Four soils representing three of the four main types of hill soil were used, although it was not possible to carry out all experiments or analyses with each soil. Experiments were carried out both in the field and in pots containing soils from the field placed either in a glasshouse or a growth room.

PART I. THE RESPONSE BY WHITE CLOVER TO THE MAJOR NUTRIENT ELEMENTS AND LIME

INTRODUCTION

The following review presents aspects of the mineral nutrition of the major nutrients which relate to the establishment and growth of white clover in hill soils. In particular it reviews the present fertilizer recommendations for hill pastures and where possible for optimum growth of white clover, and emphasises factors which affect the uptake of the major elements, nodulation and nitrogen fixation and the effects of elemental deficiency on whole plant growth and mineral composition. In addition, other aspects of nutrition that are relevant to a particular element are discussed, e.g. source of nitrogen for plant growth. Wherever possible, the effects of nutrients on the growth of white clover are quoted but information on this plant is scanty and, in cases where qualitative responses or requirements are known only in other species, these are described. Likewise, where information for plants grown in soil is lacking, relevant results for plants grown in nutrient solution are quoted. In general, the principles of plant nutrition described in the recent book by Mengel and Kirkby (1978) have been used to underpin the discussion since in my opinion it is a competent guide to the subject.

Cultivars of white clover are divided into categories based upon the size of the leaves, the small and medium small leaved varieties being recommended for use in hill

pastures (see General Introduction). Little attention in the literature has been paid to white clover, by comparison with that given to other forage legumes, e.g. Subterranean clover and lucerne. Moreover, much of the information on white clover emanates from New Zealand and is for Huia (medium small leaved) and other studies in warmer climates have used the large leaved varieties alone. The data for all the categories of white clover have been included but the response to nutrients may differ in cultivars bred for very different conditions of growth. For example, medium large leaved cultivars of white clover are more tolerant of applications of nitrogen fertilizer than small leaved cultivars when grown in mixed swards (Laidlaw, 1978). The fertilizer recommendations for establishment of white clover in reseeded pasture of all types were last reviewed by Chestnutt and Lowe (1970). These authors emphasise that management practices, including fertilization, should be directed specifically at white clover which is regarded as having a more exacting requirement than grass. If the same principles were applied by the Scottish colleges when determining fertilizer recommendations for hill land improvement as described in the next section, the fertilizer requirements of white clover would be synonymous with those for improved hill pastures.

PRESENT FERTILIZER RECOMMENDATIONS FOR ESTABLISHMENT OF HILL PASTURE (WHITE CLOVER)

The three Scottish Colleges of Agriculture (West, East and North) recommend levels of lime and fertilizers for hill land improvement (WSAC, 1975; ESCA, 1977; NSCA, 1978). The levels are given for soils with moderate levels of phosphorus and potassium but adjustments are made for soils with lower and higher levels after soil analysis. The East and West Colleges stress that soil analysis can only be a guide to liming and fertilizer policy and is best interpreted by an advisor with local knowledge.

ADAS (1976, 1978, 1979) recommends fertilizers for grassland improvement based upon soil analysis, and the levels of available nutrient in soils are divided into ten categories which are given an Index Number from 0 to 9. Hill soils are generally in categories 0-2.

A cautionary note must be sounded on the value of the routine methods of analysis of soils. The method used by the colleges is based upon the weight of soil, i.e. mg nutrient/kg soil but the ADAS analysis is based upon volume, i.e. mg nutrient/l soil. Hill soils contain greater levels of organic matter (40% or more) than arable soils (20% or less) and are consequently less dense. Assessments of nutrients in hill soils based upon the weight of the soil can therefore be misleadingly high (Pimplaskar et al., 1980).

Rates of application of each element will be discussed in turn. There have been several studies of nutrient requirements of improved pasture on deep peat (Grennan and Mulqueen, 1964 a,b,c; Reith and Robertson, 1971; Reith et al., 1973) but there have been few studies on the mineral soils.

Nitrogen

The aim of hill pasture improvement is to develop a sward supported by biologically fixed nitrogen. However, dressings of nitrogen fertilizer are generally applied when pastures are sown to provide nitrogen before biologically fixed nitrogen is available (Haystead and Marriott, 1979b). The amount of fertilizer applied should be carefully chosen to be beneficial in early establishment without being detrimental to the later growth of white clover by depression of nodulation and nitrogen fixation.

In addition to the levels of nitrogen recommended by the advisory services (Table 1), other workers have suggested levels of nitrogen required for establishment of hill pasture on peat. O'Toole (1967) found 22-45 kg N/ha was sufficient for establishment of a white clover pasture on deep peat in Ireland and Reith and Robertson (1971) suggested about 35 kg N/ha for establishment in a raised bog in Scotland; higher rates increased total pasture yield but suppressed clover. In a recent investigation on a deep peat soil, Haystead and Marriott (1979b) found that ammonium fertilizers applied at less

Table 1. The levels of lime and fertilizers recommended by the advisory services for establishment of reseeded hill pastures

Advisor	Soil status or index	Lime tonne/ha	pH required	Nitrogen kg/ha	Phosphorus kg/ha	Potassium kg/ha	Magnesium
ESCA	Low				64	91	
	Moderate	5	5.8-6.3	80	56(22)†	66	as magnesian
	High				48	41	limestone
WSAC	Low	by soil	5.0 peats		38	83	where necessary
	Moderate	analysis	5.8 mineral	40	24	50	as magnesian
	High		soils with >20% OM		10	17	limestone
NSCA	Low				85	67	where necessary
	Moderate	10	5.8-6.2	50	77(21)	42	as magnesian
	High				69	17	limestone
ADAS	0	by	5.3 peats	40	53	104	where necessary
	1	soil	6.0 mineral	0	27	62	as magnesian
	2	analysis	soils	0	17	25	limestone
Pwllpeiran EHF	-	7.5		118	235(8)	32	as magnesian limestone

† Figures in brackets are the levels of soluble phosphate fertilizer recommended

than 90 kg N/ha had little effect on grass and clover growth. They recommended that 100 kg N/ha should be applied after germination when seedlings are established, after nodules are initiated, when the plants are able to rapidly take up nitrogen.

There is a considerable variation in the amounts of nitrogen recommended as there is a 3 fold difference between the greatest and the least amount (Table 1). It could be expected that higher levels would be recommended for the west of the country where the higher rainfall would increase losses from leaching but there is no such trend. It is also surprising that recommendations do not specify the form of nitrogen to be applied, because nitrate-N is leached more rapidly from the soil than ammonium-N (Russell, 1973).

Applications of ammonium-nitrogen at about 100 kg N/ha to seedlings, after the nodules are initiated, may make the best use of nitrogen fertilizer and minimise the detrimental effects to later growth. However, a separate application of nitrogen would, in practice, have to be weighed against the extra cost of spreading the fertilizer.

Phosphorus

There is a large variation in the levels of phosphorus recommended for establishment of improved pastures (Table 1). Generally, phosphorus is applied both in soluble form for establishment and as a slower acting form. In the past basic slag was used but this

fertilizer is now unobtainable and the most likely substitute is ground mineral phosphate.

Grennan and Mulqueen (1964a) reviewed the levels of phosphorus fertilizer recommended for pasture growth on peat soils in Northern Europe prior to 1964 and found that 50-80 kg P/ha were recommended for establishment. In their own experiments on a deep peat soil in Ireland, they found that white clover did not establish with less than 40-60 kg P/ha. These levels lie within the range of recommendations from the British advisory services, but Reith and Robertson (1971) suggested 100 kg P/ha would ensure good establishment of an improved pasture on deep peat.

Potassium

Levels of potassium recommended vary to about the same extent as levels of phosphorus and nitrogen (Table 1). Similarly, recommendations do not seem to be related to the climate, e.g. levels recommended in the south are not greater than those recommended in the north where the climate is colder and the growth rate is slower. Levels recommended in the west are not greater than those recommended in the east where rainfall is lower and losses by leaching are less.

Grennan and Mulqueen (1964b) reviewed the levels of potassium fertilizer applied for establishment of pastures grown on peat in Europe prior to their own work and levels ranged from 112-180 kg K/ha. From their own

work 84-112 kg K/ha was recommended, whereas Reith and Robertson (1971) recommended 140 kg K/ha. The levels are on the whole greater than those suggested by the British advisory services.

Calcium/Lime

Lime is applied to acid soils to reduce the concentration of hydrogen ions and the solubility of aluminium and manganese and to increase the availability of calcium, and some trace elements. The relative importance of these factors varies between soils. Over-liming can reduce the availability of some essential elements, e.g. boron, zinc, copper and phosphorus (Lucas and Davies, 1961), and can increase the availability of molybdenum and sulphur which can interfere with copper in the rumen of sheep and induce deficiency of the latter element (Whitelaw et al., 1979).

The optimum pH at which a plant will grow in a soil is related to soil texture. It is lower for organic soils than mineral soils and increases in mineral soils with the clay content (Mengel and Kirkby, 1978). McNeur (1953 and 1954) found the optimum pH for growth of two strains of white clover was pH 5.5 and pH 5.3. Adams and Lowther (1970) suggested that a pH of 5.6 or 5.7 was adequate for white clover establishment and growth. Lowther (1974) grew white clover cv. Grasslands Huia in soils ranging from pH 4.7 to 6.2. Applications of lime increased the growth of clover on soils with a pH less

than 5.0. In Ireland satisfactory growth of clover was found at pH levels between 4.8 and 5.0 in a blanket peat (O'Toole, 1963). Therefore the optimum pH for clover growth lies somewhere between 5.0 and 6.0: the optimum in peat is probably nearer 5.0 and, in mineral soils, between 5.5 and 6.0.

Lime has to be applied to the soil to bring the pH to the optimum level. In many acid hill soils 15-20 tonne /ha is required to increase the pH in the top 10 cm. Such levels are not economically justified and levels from 5-7 tonne /ha, sufficient to increase the pH near the surface of the soil, are adequate for establishment and growth of clover for 5-7 years (Newbould, 1979).

Of the advisory services, the WSAC and ADAS are the most flexible in the recommendations for lime (Table 1). The variation in the recommended levels of lime (5-10 tonne /ha) is not as great as with fertilizers.

Magnesium

The consensus of opinion is that magnesium should be applied with lime as magnesium limestone (Table 1). This compound is cheap and its application requires no extra labour, although magnesian limestone may be difficult to obtain in the more remote areas.

The only reported response by white clover to magnesium fertilizer came from New Zealand where there was a response to 50 kg Mg/ha in a pumice soil very low in exchangeable magnesium (0.73 Mg/100 g soil, McNaught and Dorofaeff, 1960). The results agree with Reith (1963)

who suggested that the readily soluble magnesium content of soil must be less than 3 mg Mg/100 g for most crops to respond to magnesium fertilizers. ESCA recommend applications of magnesium in soils with up to 15 mg Mg/ 100 g to reduce the risk of hypomagnaesemia in animals, (see p.38).

There are many reports that application of other cationic fertilizers affect the uptake of magnesium by crops, the most notable being potassium (Stewart and Holmes, 1953; Reith et al., 1964), calcium,(Stenuit, 1959; Hall, 1971), and manganese (Lohnis, 1960; Maas et al., 1969). Manganese can substitute for magnesium in many enzymic reactions, but the competitive effects between magnesium and calcium or potassium seem to be associated with the balance of ions in the plant (Mengel and Kirkby, 1978).

In acid soils, hydrogen or aluminium ions may inhibit magnesium uptake and, on limed soils, calcium may inhibit the uptake of magnesium by plants. Stenuit (1959) suggested from experiments with oats that at around pH 5 cation antagonism is at a minimum. Reith (1963) suggested that potassium levels necessary to maintain yield have little effect on the magnesium content of either grasses or clovers, but greater applications of potassium may depress the magnesium content of the herbage.

The following two sections review factors of nutrient uptake and assimilation, nodulation and nitrogen fixation which could influence the response by white

clover to applied nutrients.

THE UPTAKE OF NUTRIENTS

Theories of ion uptake are not discussed here but Dainty (1962), Baker and Hall (1975) and Bowling (1976), amongst others, have recently written excellent accounts. In addition to the uptake of nitrogen, it is also necessary to discuss the assimilation of different forms of nitrogen because of interactions with other elements.

Nitrogen

In grassland soils mineralised nitrogen is predominantly in the form of ammonium (Richardson, 1938). Levels of ammonium in grassland at Rothamsted range between 3 and 9 ppm and similar levels were reported by Floate (1970) of 10-15 ppm NH_4 in unimproved hill soils with 0-1 ppm NO_3. This contrasts with arable soils where nitrate is the predominant form (Russell, 1973). The reason for the difference is not clear but washings from the roots of Lolium perenne have been shown to inhibit nitrification (Moore and Waid, 1971). Low temperature and acidity also are known to inhibit nitrification (Russell, 1973) and may further contribute to the low level of nitrate in hill soils. The inhibition of nitrification thus protects the nitrogen from losses through leaching and denitrification. However, fertilizer applied as nitrate is not so protected and on the hill, where rainfall is high, the fertilizer may be leached. In waterlogged soils where plant growth is retarded, nitrate will also be

be lost through anaerobic denitrification provided the pH is not too low (Broadbent and Clark, 1965).

The three forms of nitrogen, nitrate, ammonium or biologically fixed nitrogen have distinct effects upon plant growth and cation uptake and will be discussed. There is a great deal of information which relates the effects of form and level of inorganic nitrogen fertilizer to the growth of plants (Kirkby, 1969; Raven and Smith, 1976). Much of the work has been conducted on single plants, over short periods of time, in nutrient solutions where the levelsof inorganic nitrogen in the nutrient media remains constant and where the pH changes in the media which results from the treatment are regularly re-adjusted to a constant level (Kirkby, 1969). Some of this work is discussed here because it is applicable to work undertaken in this study.

Raven and Smith (1976) described the effects of assimilation of nitrate, ammonium and N_2 in relation to the disposal of toxic by-products, H^+ or OH^-. Ammonium is mainly assimilated in the root and at least one excess H^+ is produced per N assimilated. The cell has no long term storage mechanism for hydrogen ions and most of the excess H^+ is excreted into the soil solution. Assimilation of one nitrate ion produces about one excess hydroxyl ion, and the charge is transferred to an organic anion when nitrogen is assimilated in the shoot and is stored in the cell vacuole with a counter cation. The organic anion/inorganic cation may be translocated to the

root where the anion is broken down to a neutral product and bicarbonate is excreted to the external solution, but the extent of carbonate excretion may depend on species and the level of nitrate (Kirkby and Knight, 1977). Biologically fixed nitrogen produces 0.1-0.2 H^+ per N assimilated, the hydrogen being excreted into the external solution probably through the root rather than the nodule.

Acidification of the soil around the plant root, when the nitrogen supply is in the ammonium form or when N_2 is fixed, may affect the solubility of other elements and cause secondary effects on plant growth. Riley and Barber (1971) found differences of 1.9 and 1.5 pH units in the rhizocylinder of soybean which was fed NO_3-N or NH_4-N when the bulk of the soil was pH 5.3 and 6.3 respectively. The pH value of the rhizocylinder affected the solubility and availability of phosphate. Dekock and Kirkby (1969) claimed that pH changes of the growth media associated with nitrogen nutrition affected the availability of iron.

The cation concentration of plants grown on NO_3-N, when assimilation occurs in the leaves, as in white clover cv. S100 Nomark (Copeland and Pate, 1969), is often greater than in plants grown on NH_4-N (Coic and Lesiant, 1971). Ammonium nutrition also depresses cation uptake in some cases because the NH_4^+ ion is a better competitor for uptake than, for example, Ca^{2+}. Nitrate nutrition stimulates cation uptake because the anions produced

when nitrate is assimilated require a counter ion to neutralize them. Ionic balance sheets calculated by Kirkby (1969) for whole tomato plants support the concepts outlined above. Legumes supplied with biologically fixed nitrogen should contain fewer cations than plants fed with NO_3-N.

It is a common observation that plants, particularly dicotyledons when fed with NO_3-N, grow better than those fed with NH_4-N (Kirkby, 1969). The reasons for this are not fully understood (Mengel and Kirkby, 1978) but, where the pH of the external media remains constant, there is little difference in plant yield with either source provided the yield was less than the maximum (Andrew and Johansen, 1978).

Phosphorus

Phosphate is actively taken up against an electrochemical gradient by plant roots (Russell and Barber, 1960). Hai and Laudelout (1966) found that phosphorus uptake was pH dependent and related to the shift in $H_2PO_4^-/HPO_4^{2-}$ ion ratio in solution and they suggested that only $H_2PO_4^-$ ions were absorbed actively. Dunlop and Bowling (1978a and 1978b) have evidence for a phosphate pump in white clover which is operated between pH 3.5 and 8.0 with an optimum uptake at pH 4.3, but they did not find a relationship between uptake of phosphorus and the concentration of phosphate ions in solution.

Potassium, Calcium and Magnesium

Potassium is actively taken up by plant roots and can influence the uptake of other cations, particularly

calcium and magnesium (Kirkby, 1979). Kirkby (1979) suggests the mechanism of competitive uptake of cations may involve attraction of cations to a non-specific anion equivalent in the cell; the cation taken up the fastest would be favoured. Potassium would be successful because it is taken up actively, unlike calcium which may only be taken up actively at low concentrations (Maas, 1969).

Another factor which may affect competition between cations for uptake is the site of absorption of ions in the root. Russell and Clarkson (1976) reported that potassium was taken up along the whole root length of barley and marrow, whereas calcium uptake was confined to the young parts of the root. The site of magnesium uptake is similar to that of calcium (Mengel and Kirkby, 1978). The differences are probably related to the path the ions take to the xylem. Potassium may move through the cytoplasm of root cortical cells and pass through the suberised endodermis (the symplastic route). Calcium and magnesium however may pass through the free space (the apoplast) and only cross the endodermis in the young part of the root before it is suberised (Russell and Clarkson, 1976).

THE EFFECT OF FERTILIZERS ON NODULATION, NITROGEN FIXATION AND THE GROWTH OF RHIZOBIA

Nitrogen

Although the aim of hill pasture improvement is to establish a sward entirely dependent on symbiotically

fixed nitrogen, fertilizers are often applied at sowing to help establishment of grasses and to provide nitrogen for the clover plant after the seed reserves are depleted and before nitrogen is available from fixation (Haystead and Marriott, 1979b). Nitrogen fertilizer is usually applied as a salt containing nitrate or ammonium or both, and the forms of nitrogen applied may affect nodulation and nitrogen fixation differently. Mineralisation of nitrogen in hill soils is slow but what is present is not nitrified from ammonium to nitrate (see p. 20). The effect of form of nitrogen and the effect of nitrogen fertilizers applied to white clover in the field will be considered.

The effect of combined nitrogen on nodulation and nitrogen fixation by legumes has frequently been reviewed (e.g. Fred, Balwin and McCoy, 1932; van Schreven, 1958; Stewart, 1966; Gibson, 1976; Munns, 1977a; Sprent, 1979). Many of the effects of combined nitrogen can be explained in terms of carbon:nitrogen ratio of the plant (Stewart, 1966) because assimilation of combined nitrogen reduces the carbohydrate available for nodule formation and nitrogen fixation (Wilson, 1940; Small and Leonard, 1969), although products of nitrate reduction in the plant may depress nitrogen fixation in the nodules (Rigaud, 1976). Thus, because nitrate and ammonium are assimilated in different ways (Hewitt and Smith, 1975), they may affect nitrogen fixation differently, but this

is not well understood because most studies have concentrated only on the effect of nitrate on nitrogen fixation (Sprent, 1979).

There may be differential effects between nitrate and ammonium on nodulation as well as on nitrogen fixation. Gibson (1959) found that ammonium-N delayed nodulation in white clover cv. S100 at low light intensity but not at full light, nitrate-N delayed nodulation at both light intensities, and a specific inhibitory effect of nitrate on nodulation was proposed. Experiments where nitrate was applied locally to roots often resulted in inhibition of nodulation at the site of placement but not elsewhere on the root system (Wilson, 1917; Harper and Cooper, 1971), which suggests there is an additional specific and local effect of nitrate upon nodulation. A possible explanation is that nitrite, formed from nitrate by rhizobia in the rhizosphere, interferes with the activity of indole-3-acetic acid during infection (Tanner and Anderson, 1963), although Gibson and Pagan (1977) grew T. subterranean with a nitrate-reductase deficient strain of Rhizobium and the normal parent strain and found no evidence that nitrite specifically inhibited nodulation.

Gibson and Nutman (1960) suggested that the growth stimulation of roots after a small amount of mineral-nitrogen was applied provided more sites for nodulation to occur when the nitrogen supply was depleted. However, the effect of low levels of combined nitrogen on nodulation and nitrogen fixation can vary with cultivar and strain of

Rhizobium (Pate and Dart, 1961; Gibson, 1976).

There is some information which relates applications of nitrogen fertilizer to nodulation and nitrogen fixation by white clover in the field. A ryegrass/white clover (cv. S184) pasture on deep peat was given 0-120 kg NH_4-N/ha before sowing, at sowing, and after nodule initiation. Measurements of yield, nodulation and nitrogen fixation (by the acetylene reduction assay) were made 123 days after sowing (Haystead and Marriott, 1979b). Nitrogen fertilizers had no effect on the numbers of nodules, acetylene reduction or yield when applied before sowing. Nitrogen applications at sowing did not affect nodule numbers or yield but stimulated acetylene reduction at 30 and 60 kg N/ha. When nitrogen was applied after nodule initiation, yield was unaffected: nodulation was depressed with nitrogen levels above 30-60 kg N/ha and acetylene reduction was enhanced at 30-60 kg N/ha but depressed at 90-120 kg N/ha. Moustafa *et al*. (1969) measured the rate of acetylene reduction on three occasions 3 to 4 months after sowing a pure sward of white clover cv. Grasslands Huia which had received none or 80 kg N/ha. The rate of acetylene reduction by clover which received 80 kg N/ha was 29-39% of that which was unfertilized. Masterson and Murphy (1976) applied about 65 kg N/ha (250 kg calcium ammonium nitrate/ha) on an established ryegrass/white clover pasture in spring. The fertilizer reduced the rate of acetylene reduction compared with an unfertilized sward until after flowering in July. Sears *et al*. (1965) found that nitrogen

fixation in pasture decreased, measured on an area basis, as the soil nitrogen level increased in a four-six year old pasture. There were two reasons given for the effect: one was the direct effect of soil nitrogen on nitrogen fixation and the other the increased competition by grasses which reduced the amount of clover in the pasture. The most important factor which governed the nodule number of white clover in a mixed sward was the amount of root material present (Young, 1958), nitrogenous fertilizers reducing the root weight and consequently the number of nodules.

Phosphorus

When legumes are fertilized with phosphorus, the increase in phosphorus in the plant is usually accompanied by an increase in nitrogen and this is true of white clover (Cullen et al., 1965; McNaught and During, 1970). The increase in nitrogen in the plant may be due to one or more factors, including earlier nodulation, more nodules, longer duration of nodules and increased efficiency of nitrogen fixation (Andrew, 1977). It is not clear whether phosphorus directly stimulates nodulation and nitrogen fixation or whether they are indirectly enhanced by improved plant nutrition and photosynthesis (Andrew, 1977; Munns, 1977a).

Potassium

Mengel and his co-workers have examined the effects of inadequate potassium supply on nodulation and nitrogen

fixation in Vicia faba and Medicago sativa and postulated that potassium deficiency reduced nodulation and nitrogen fixation by reducing the energy supply (Mengel, Haghparast and Koch, 1974; Feigenbaum and Mengel, 1979). No similar work has been done with white clover.

Calcium/Lime

This section pays particular attention to the effects of calcium and acidity on nodulation and nitrogen fixation in legumes because hill soils are very acid. In solution culture, the two effects can be separated but application of lime in the field to reduce acidity results in an increase in calcium in the soil.

Munns (1977b; 1978) has related the effects of four factors of soil acidity which can reduce plant growth (low pH, calcium deficiency, manganese and aluminium toxicity), to the growth, nodulation and nitrogen fixation of legumes. From published information he grouped species of legumes into the categories highly tolerant, moderately tolerant, moderately sensitive and highly sensitive to acidity. White clover was in the highly sensitive group with Medicago species and Pisum sativum but it was concluded that the behaviour of one legume cannot be predicted from experience with another.

Symbiotically dependent legumes are generally more sensitive to soil acidity than the same legume fed with nitrogen fertilizer (Munns, 1978). Andrew (1976) in sand culture experiments confirmed this for a Grasslands Huia white clover. The pH of the media varied from

4.0-6.0. When the yield of plants at pH 4.0 was compared with that at pH 6.0, growth was depressed by 80% when symbiotically dependent but was only depressed by 20% when fed with nitrogen fertilizer.

Generally roots are less sensitive to low pH and calcium concentration than nodulation. This has been demonstrated for Medicago sativa (Munns, 1968), Pisum sativum (Lie, 1969), and Trifolium subterraneum (Lowther and Loneragan, 1968). Andrew and Norris (1961) grew white clover in a gley soil, pH 5.5 and low in calcium. Applications of calcium carbonate up to 753 kg/ha changed the pH of the soil by up to 0.2 units and increased the base saturation by calcium 10 fold. Applications of calcium carbonate produced a 5 fold increase in root and nodule dry weight but nodule numbers increased about 20 fold. The weight of nodules per unit weight of root remained constant in all calcium treatments indicating that infection was more sensitive to calcium than root or nodule growth. Munns and Fox (1977) found similar results when growing white clover cv. Louisiana in a Hawaiian oxisol at pH 4.7-7.1. Nodule dry weight remained constant through the pH range but the number of nodules increased with pH. Dart (1976) reported that limitation of nodule number by acidity can be compensated by an increase in size.

The most acid sensitive process in nodulation is infection and it coincides with the curling of the root hair (Munns, 1978) and is the rationale behind lime

coating of legume seed. The lime coat increases the pH in the soil around the germinating seed and allows infection and, from then on, nodulation and further development of the legume are less sensitive to acidity. Calcium can moderate the harmful effect of low pH. O'Toole and Masterson (1968) found that nodulation of white clover cv. S100 Nomark grown in a blanket bog of pH 4.3 was enhanced by greatly increasing the soil calcium content at the same pH.

There is evidence for some legumes that the better nodulation as a result of applying lime cannot fully account for the increase in yield and/or nitrogen assimilation (Munns, 1978). Andrew (1976) reported for white clover cv. Grasslands Huia that maximum nodulation occurred at pH 5.0 and above, but growth and nitrogen yield in shoots had not reached maximum at pH 6.0. Similar results were obtained by Munns et al. (1977) with white clover cv. Louisiana.

The calcium requirements for growth of some strains of Rhizobium trifolii in artificial media is 0.025 mM with an additional requirement of 0.5 mM divalent cations (Vincent, 1962). Levels of calcium and magnesium in the soil as a whole may be sufficient to fulfil these needs but there may be deficiencies in the rhizosphere. There is evidence for legumes other than white clover that rhizobial growth rates on roots or root washings from legumes respond to increases in pH or calcium (Munns, 1968; Lie, 1969; Lowther and Loneragan, 1968). The

conditions for rhizobial growth have only been evaluated for a few strains and it is possible that there is wide variation of growth requirements in different strains.

Magnesium

The growth of rhizobia is inhibited in media with less than 0.1 mM magnesium (Vincent, 1962). It is difficult to compare nutrient requirements in artificial media to those in the soil, but hill soils generally have magnesium levels greater than 50 mg/100 g dry soil (Reith, 1973) (approximately equivalent to 20 mM/kg soil), and it is unlikely that growth of rhizobia will be inhibited by deficiency of magnesium. Magnesium has no documented effect on nodulation and nitrogen fixation (Munns, 1977a; Andrew, 1977).

In the final section the levels of nutrient in white clover and the nutrient deficiency symptoms are discussed.

LEVELS OF NUTRIENT IN THE PLANT AND DEFICIENCY SYMPTOMS

The relationship between plant growth and the concentration of nutrient in the tissue is illustrated in Fig. 2. The nutrient content in the tissues is low when plant growth is severely restricted by deficiency of the element (a) but where there is a little more nutrient available for growth the concentration in the tissue decreases a little through dilution (b). Plant growth increases rapidly to the maximum (d) with a small change in tissue concentration (a) to (d). The least concentration of nutrient in the tissue above which there is no

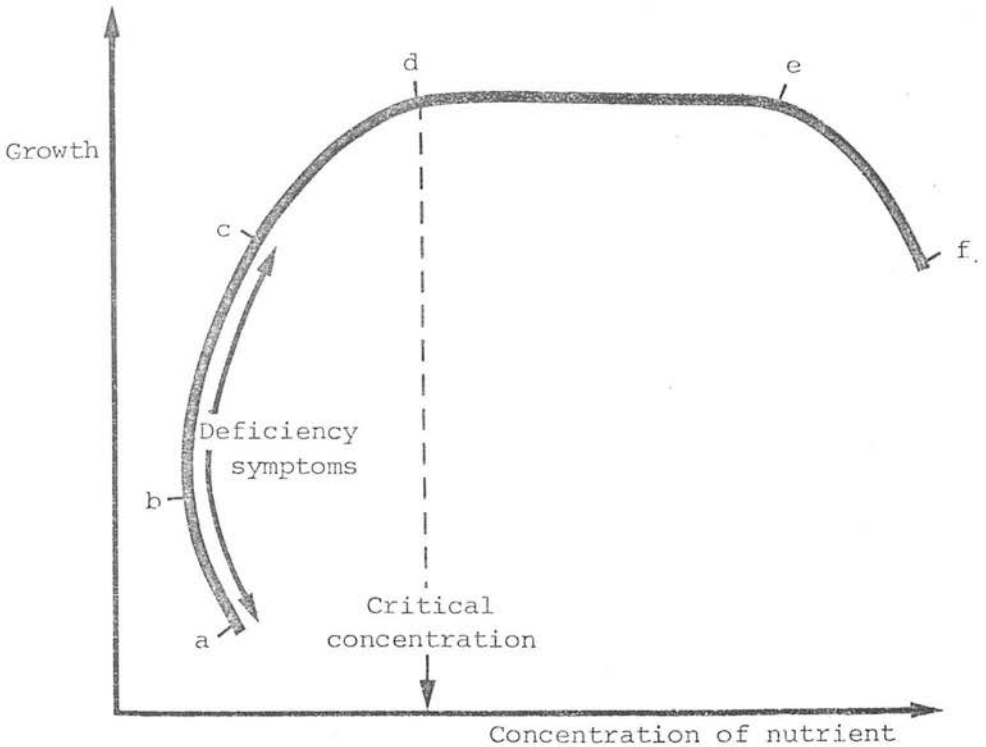


Figure 2. The relationship between plant growth and the concentration of nutrient in the tissues (after Smith, 1962).

increase in growth (d), the critical concentration (Ulrich, 1952) is of agronomic importance. Deficiency symptoms generally appear at a lower concentration than the critical concentration and there is a range where there are no visual symptoms but where growth is limited by deficiency of the nutrient (c) to (d).

The range of concentration greater than the critical one where plant growth is static (d) to (e), often called the 'luxury range' (Macy, 1936; Smith, 1962; Loneragan and Snowball, 1969; Mengel and Kirkby, 1978) but White (1979) refers to it as the 'optimum range'. Very high levels of nutrient in the tissue can be toxic (e) to (f), but, unlike the other authors, White (1979) refers to this depression in growth at high concentration of nutrient as the 'luxury range'. In this thesis, where the 'luxury range' is mentioned, it refers to the range where the nutrient concentration increases and growth remains static (d) to (e).

'Critical concentrations' in plant tissues may vary depending upon the maturity of the plant and upon the tissue analysed (McNaught, 1958). In the discussion below, critical concentrations of white clover leaf and petiole during vegetative growth are quoted, although fluctuations in the nutrient content of white clover are small compared with other herbage species (Whitehead, 1966; Spedding and Diekmahns, 1972). In legumes, the source of nitrogen, mineral or biologically fixed, may affect the critical concentration and the values quoted

are for plants which derived most nitrogen from fixation. The mobility of ions in the plant can also affect the critical concentration and will be referred to in the section on calcium.

There is little published information about the critical concentrations of nutrients in small leaved cultivars of white clover grown in British conditions. However, Andrew (1960) in a study using a large leaved cultivar of white clover, 'Irrigated White', in subtropical conditions, identified critical concentrations for phosphorus and potassium. The information he obtained is compared with other information in the following review of levels of nutrient in the plant and deficiency symptoms.

Nitrogen

The nitrogen content in white clover varies from about 2.7-5.3% and the concentration decreases with advancing maturity (Spedding and Diekmahns, 1972). The concentration of nitrogen in herbage below which growth is limited, i.e. the critical concentration (Ulrich, 1952), has not been determined, probably because in most circumstances nitrogen fixation provides sufficient nitrogen for growth when there is insufficient nitrogen from the soil. However, in hill soils where levels of available nitrogen are low (Floate, 1970) and where the few rhizobia present in the soil are often ineffective at nitrogen fixation (Holding and King, 1963), plants may become deficient in nitrogen.

Symptoms of nitrogen deficiency first appear in the older leaves: leaves become chlorotic and growth is restricted, but deficiency has to be very severe before the leaves begin to die (Wallace, 1961). Other nutrient deficiencies produce similar symptoms to nitrogen, e.g. iron and sulphur deficiency results in chlorosis of the young leaves, and therefore care must be taken in diagnosis of a particular nutrient deficiency from leaf symptoms alone.

Phosphorus

According to the survey by Spedding and Diekmahns (1972), the range of phosphorus concentration in the shoots of white clover lies between 0.19 and 0.47%. The critical concentration of phosphorus for white clover occurs around 0.20-0.25% dry matter under conditions in Australia and New Zealand (Andrew, 1960; McNaught and During, 1970; Jackman and Mouat, 1972).

There are no distinctive symptoms of phosphorus deficiency: growth is restricted, stems and petioles are thin and leaves become a dull bluish-green compared to healthy plants (Wallace, 1961), and it would be difficult to diagnose phosphorus deficiency in the field. In very severe cases of deficiency, the stems become red.

Potassium

The range of potassium levels in white clover lie between 1.54 and 3.8% according to Spedding and Diekmahns (1972). The critical level of potassium in the shoot of

white clover was reported to be 0.8% for Ladino clover by Ulrich (1945), 1.8% by McNaught (1958) and 1.1% by Andrew (1960) in subtropical Australia. Potassium is the element which is usually associated with 'luxury uptake'. From the data reported above, two to four times as much potassium can be present in the tissues than is required for maximum growth. Examination of maximum levels of phosphorus, calcium and magnesium presented here indicate that luxury uptake of these elements is in a similar order of magnitude.

Potassium deficiency symptoms in white clover are very distinctive - they appear as white marks around the margins of the older leaves, the marks form into brown necrotic areas and the leaf dies (Wallace, 1961). Plants which are deficient in potassium have a higher dry matter content, are less resistant to drought, frost and disease than healthy plants (Mengel and Kirkby, 1978).

Calcium

The calcium content in white clover varies from about 1.2-2.3% (Spedding and Diekmahns, 1972) and may reach 3% (Whitehead, 1966).

Problems arise in the determination of the critical concentration of calcium in the tissue because, unlike the other elements discussed, calcium is not mobile in the plant. If the level of calcium in the growth medium fluctuates greatly, as it can do in experiments where plants are grown in solutions, the older leaves may contain luxury levels while the meristematic tissues are

deficient (Loneragan and Snowball, 1978). However, in soils, the level of calcium may increase abruptly when they are limed or fertilized but levels will fall gradually as most calcium salts are relatively insoluble and calcium ions are held on the exchange sites and are not liable to be leached rapidly (Russell, 1973; White, 1979). Therefore, in soils, levels of calcium in the shoots will decrease slowly and an approximate level where growth is limited by calcium could be determined for diagnosis. Alternatively, the meristems could be dissected from the plant and analysed separately from the mature leaves to get a true critical concentration, but preparation of material for analysis would be time consuming and special techniques would have to be used to analyse small samples of meristematic tissue.

When calcium carbonate was added to a humic gley soil of pH 5.5 grossly deficient in calcium (exchangeable calcium 4 mg/100 g) in a pot experiment, increases in growth were associated with increases in calcium content of herbage from 0.24 to 1.02%, and an increase in soil pH from 5.5 to 5.7 (Andrew and Norris, 1961). While it is appreciated that interactions between calcium and hydrogen ions on nodulation and nitrogen fixation can confound responses to calcium (see p.29), the plants in this experiment were grown on soil near to the optimum pH quoted for white clover (see p.17) and, because the application of calcium carbonate only slightly increased the pH of the soil, it is suggested that 1% Ca in dry

matter may be near the concentration of calcium in whole shoot below which growth may be limited by calcium when grown in soil.

When white clover plants are deficient in calcium, growth is restricted and young shoots begin to wilt and die. The margins of young leaves become chlorotic or scorched (Wallace, 1961).

Magnesium

The magnesium level in white clover lies in the range 0.15-0.29% dry matter (Spedding and Diekmahns, 1972). McNaught and Dorofaeff (1965) suggest that the critical concentration of magnesium in the dry matter of white clover is 0.12%. There is little seasonal change in the magnesium content of white clover herbage. In contrast, the content of grasses is least in spring and most in autumn (Reith, 1963). When the magnesium content of herbage is below 0.2% dry matter and the potassium content is above 3% and the nitrogen level is high, the deficiency disease in grazing animals, hypomagnesaemia, is likely to occur (Wolton, 1963). The magnesium content of grasses does not often reach this level until summer and a high proportion of clover in pastures is necessary to keep up the overall magnesium content. However, white clover begins growth later in the season than most grasses and magnesium levels in herbage in spring may be less than 0.2% (Todd, 1961). Applications of nitrogen fertilizers to pastures in spring will reduce the clover and the

magnesium content and increase the nitrogen content of the herbage and therefore the likelihood of hypomagnesaemia (Reith, 1963).

Magnesium is mobile in the phloem (Steucek and Koontz, 1970) and deficiency symptoms first occur in older leaves. In white clover the symptoms are reddish bands around the margins of the leaves, with inter-veinal chlorosis: later the whole leaf turns reddish brown and dies (McNaught and Dorofaeff, 1960).

PLAN OF EXPERIMENTS

The four areas of the nutrition of white clover reviewed here indicate a lack of precise knowledge for this species. Since the establishment and persistence of white clover is the key to successful hill pasture improvement and is vital for an increase in economic returns from hill farming, emphasis in experimentation was placed on the responses to the major nutrient elements and lime, and the interactions between them. The data also identified levels of nutrients in the shoot below which growth was limited. These values are important for experimental interpretation and may assist in diagnosis of deficiency and indicate levels of fertilizer needed to maintain existing clover-rich swards.

Therefore the experiments were planned to investigate the following aspects: responses to nitrogen, phosphorus and potassium (Experiments 1-4); responses to lime and magnesium (Experiments 5-6); and the interaction between lime and phosphorus (Experiment 8). A description of the methods which were used follows.

MATERIALS AND METHODS

LABORATORY EXPERIMENTS

Fertilizer treatments

Fertilizer treatments for individual experiments are described in experimental section with the results. All levels of fertilizers in the pot experiments are expressed in terms of kilograms of the nutrient element per hectare for ease of comparison with the field trials. The amounts of chemical applied to pots were calculated on an area basis and it is appreciated that extrapolation from mg/pot to kg/ha is approximate. Quantities of chemicals applied, expressed as the weight of the nutrient and of the compound applied to each pot, and as the equivalent in kg/ha, are given in Appendix 1. Table 2 summarises the treatments applied to pot experiments.

Soils

The three soils used were a brown earth (Sourhope series), collected from the Sourhope Research Station, Roxburghshire, a dry peat from the Glensaugh Research Station, Kincardine, and a deep peat collected from the Lephinmore Research Station, Argyll. The dry peat consisted of the layer of peat (15-20 cm deep) from the surface of a peaty podzol.

The soil pH, loss on ignition, bulk density and values for nutrients extractable in ammonium acetate (pH 4.5) (Van den Hende *et al.*, 1952) are given in Table 3. Extractable nutrients are related both to soil volume and weight because the densities of the soils are very

Table 2. A summary of the fertilizers given in the pot experiments (a more detailed account in Appendix I)

Experiment	Fertilizer applications (kg/ha - unless otherwise stated)								
	N (as $Ca(NO_3)2$)	P Level	P Chemical	K Level	K Chemical	Lime (as $CaCO_3$)	Mg Level	Mg Chemical	S (as $MgSO_4.7H_2O$)
1	0 20 40 80	0 40 80 160	$Ca_3(PO_4)_2$	0 40 80 160 320	KCl	4323 6005 including $CaCO_3$ equivalent of MgO	677	MgO	None
2	None or 200 ppm	0 40 160	$Ca_3(PO_4)_2$	0 40 160	KCl	2715	10	$MgSO_4.7H_2O$	None
5	None	150	$Ca_3(PO_4)_2$	300	K_2SO_4	1370 brown earth 3470 peat	0 10 100	$MgSO_4.7H_2O$	123 136 255
6	None	145	$Ca_3(PO_4)_2$	0 76 152 304	KCl	0 1450 2900 5800	0 36 72 143	$MgSO_4.7H_2O$	0 46 93 187
7	None	145	$Ca_3(PO_4)_2$ or $Ca(H_2PO_4)_2.H_2O$	304	KCl	0 1450 2900 5800	None		None

Table 3. Some characteristics of the soils used in the experiments

Soil	pH	Loss on ignition (%)	Bulk density (g/cm^3) 0-10cm depth	Ammonium acetate extractable nutrients $mgl^{-1}/mg100g^{-1}$						
				K	Ca	Mg	P	Al	Fe	Mn
Sourhope Brown earth	4.3	25	0.65	486/75	307/47	167/26	39/6	116/18	14/2	43/7
Glensaugh Dry peat	4.3	70	0.19	57/30	38/20	38/20	4/2	40/21	6/3	6/3
Lephinmore Peat	3.8	86	0.10	15/15	69/69	37/37	1/1	15/15	12/12	7/7

different. The elements in the extracted solution were measured by standard methods (Allen, 1974); potassium by flame emission spectrophotometry; calcium, magnesium, iron and manganese by atomic absorption spectrophotometry; phosphorus by a molybdenum blue method; and aluminium by an alizarin red S method (Lancaster and Balasubramaniam, 1974).

Nutrients extractable in ammonium acetate were measured because the method is used routinely by the Scottish Colleges and because the analysis was required to emphasise differences between soils and not to measure the absolute values of nutrient in the soil which are best equated with plant growth. Values for aluminium, iron and manganese in the peat do, however, indicate that the levels of these elements in the soil are unlikely to be toxic to plant growth (Sheppard, 1980). In experiments where the brown earth was used, the soil was limed to pH 5.5 or more, at which pH evidence suggests that soluble and exchangeable aluminium, manganese and iron are unlikely to be toxic (Sheppard, 1980).

The peat was partially dried and shredded before use; the brown earth was passed through a 6 mm sieve. All of the pot experiments except one (Experiment 2) were carried out in 10 cm diameter plastic plant pots with 250 g deep peat (approx. 30% DM), 250 g dry peat (approx. 42% DM), or 400 g brown earth (approx. 60% DM) per pot. In Experiment 2, 7.5 cm diameter plant pots were used with 120 g deep peat per pot. The experiments were housed in the HFRO

glasshouses or growth rooms (Experiment 2) at Bush Estate, the Lothians.

Plants

The cultivar of white clover 'New Zealand Grasslands Huia' was used in all the experiments. Forty seeds were sown into the 10 cm diameter plant pots; in the 7.5 cm diameter plant pots 20 seeds were sown and the seedlings were thinned to ten healthy specimens.

Rhizobia

Inoculum was supplied by the Microbiology Department, Edinburgh School of Agriculture. A suspension of three strains of Rhizobium was given to the soil in each pot one week after the seeds germinated, which provided a minimum of 1000 cells per seedling.

Trace elements

A solution of trace elements was applied to the pot experiments and the rates of application were:-

Copper	2.55 kg/ha	as cupric sulphate
Cobalt	0.42 kg/ha	as cobalt sulphate
Zinc	0.45 kg/ha	as zinc sulphate
Manganese	2.33 kg/ha	as manganese sulphate (peats only)
Boron	1.02 kg/ha	as solubor
Molybdenum	0.88 kg/ha	as sodium molybdate

The sulphates were dissolved together: the solubor and sodium molybdate were dissolved separately. The solutions were acidified to approximately pH 3 with hydrochloric acid to avoid precipitation when the solutions

were mixed together. The complete solution was diluted until there was sufficient to apply 10 ml to each pot. The solution was applied to the saucer of the pot.

Watering

A saucer was placed beneath each pot and deionised water was poured into the saucer daily, or more often when necessary to keep the soils fairly moist. For the first month after an experiment was sown, while the seedlings established, the surface of the soil in the pot was also sprayed with deionised water.

Lighting and heating

In the glasshouse, plants grown at times of the year when the daylength was less than 16 hours were given supplementary light at a mean of 68 W/m^2, with a range of 53-82 W/m^2, to extend the daylength to 16 hours. The minimum temperature in the glasshouse was held above 13^0C throughout the year.

Growth rooms were held at 15 ± 1^0C day / 10 ± 1^0C night temperature with 250 ± 10 W/m^2 light intensity (70% photosynthetically active light) for 16 hours and 90% humidity. Photosynthesis by white clover plants, of approximately the size of those in pot experiments described here, measured by an infra red gas analyser, is saturated by a light intensity of about 200 W/m^2 (Lamb, personal communication).

Replication and experimental design

There were three replicated blocks in Experiments 1,

6 and 7 and five replicated blocks in Experiments 2 and 5. Treatments in all experiments except the first and fifth were completely randomised within blocks. Treatments in the first were arranged in a cyclic design (see Experiment 1) and, in the fifth, in a split pot design.

Measurements

The number of seedlings established 10-14 days after germination were counted in some experiments. The dry matter of shoots was measured after drying for 12 hours at 80^{0}C. The content of major elements in the shoots except for nitrogen was measured by X-ray fluorescence spectrometry (Evans, 1970). Nitrogen was determined by a modification of the method described by Allen (1974) following Kjeldahl digestion using mercury as the catalyst.

In some experiments the root dry weight was measured and the number of nodules were counted.

Statistical analysis of the data

Experimental results were analysed using analysis of variance and correlation (Snedecor, 1965). The statistic used in this thesis to indicate (a) differences in measured values caused by treatment, is the 'standard error of difference between means' (SED) and (b) correlation, is the correlation coefficient.

The statistical programmes used to analyse data were (1) EDEX (ARC Unit of Statistics, Edinburgh), (2) Varanal (J. Rogers, HFRO), and (3) Multreg (Edinburgh Regional Computing Centre).

In the text, all effects of treatment mentioned are significant to a probability of at least 5% unless otherwise stated.

FIELD EXPERIMENTS

Two field experiments are described, both of which were sited on the deep peat at the Lephinmore Research Station, Argyll.

Treatments

The fertilizers applied to the trials are listed in Table 4.

Site preparation

Both experiments were set up on areas of established ryegrass/white clover pasture. The procedures used to establish these pastures were essentially those described by NSCA (1972) and will be described in the materials and methods section in Part II where sites were prepared from indigenous pasture. Topdressed fertilizers were broadcast on the soil surface.

Plants

The cultivar of white clover was 'New Zealand Grasslands Huia', and of ryegrass was 'Perma'.

Replication and experimental design

Experiment 3 had three replicated blocks and Experiment 4 had four replicated blocks. Treatments were completely randomised within blocks.

Table 4. The fertilizers applied in the field

Experiment		Fertilizer applications (kg/ha) N as nitram	P as single superhphosphate unless otherwise stated	K as muriate of potash	Trace elements solution prepared as on page 45	Lime/Mg as magnesian limestone
3	1972	30	60 as basic slag	75	applied	5000
	1976	none	40	80	applied also 0.5 kg Mo/ha	2500
4	1974	0 or 30	20 + 20 as slag	60	applied	7000
	1975-77		40 annually	40 annually		
	1978	none	0 30 40 50	0 50 100 150	none	none

Measurements

The dry matter yield of the pasture was measured, from quadrats placed at random in each plot, by cutting the herbage (above 2 cm) using Wilkinson Sword cordless shears. In the laboratory each herbage sample from the field was thoroughly mixed and a subsample (1/10th the fresh weight of the field sample) was taken for separation into three components: white clover, ryegrass and indigenous species. The remainder of the sample taken from the field and the separated subsample were dried at 80^{0}C for 24 hours (or longer where necessary), weighed and the percentage of each component in the subsample was applied to the total dry weight.

The following section describes the experimental work aimed at measuring the effects of added nitrogen phosphorus and potassium on the growth of white clover in three hill soils with particular emphasis on the deep peat.

THE EFFECT OF NITROGEN, PHOSPHORUS AND POTASSIUM ON THE YIELD AND NUTRIENT CONTENT OF WHITE CLOVER

Nitrogen, phosphorus and potassium fertilizers are usually applied to the seedbed just before the seed is sown in agricultural practice. It was decided firstly to investigate (a) the responses to these three elements in soil given adequate lime and magnesium fertilizer; (b) any growth interactions between nitrogen, phosphorus and potassium; (c) any effects of nitrogen, phosphorus or potassium treatment on the calcium and magnesium content of the herbage; (d) the effect of phosphorus and potassium on the nitrogen content of the herbage; (e) the amount of nutrient provided by the soils; and (f) the efficiency with which fertilizer was absorbed from the soil.

EXPERIMENT 1. Nitrogen x phosphorus x potassium factorial experiment on three hill soils

EXPERIMENTAL

One soil from three of the four main groups of hill soils was used in the experiment and these were the Sourhope brown earth, the Glensaugh dry peat and the Lephinmore peat. Four levels of nitrogen, 0, 20, 40, and 80 kg N/ha were applied with four levels of phosphorus 0, 40, 80 and 160 kg and five levels of potassium 0, 40, 80, 160 and 320 kg K/ha in a complete factorial combination. The elements nitrogen, phosphorus and potassium

were supplied as calcium nitrate, tricalcium orthophosphate and potassium chloride. A basal dressing of lime at 4.32 tonnes/ha was given as calcium carbonate with 0.68 tonnes/ha of magnesium oxide. The magnesium oxide, in addition to supplying magnesium, had a neutralizing effect equivalent to 1.68 tonnes calcium carbonate. Trace elements and rhizobia were applied as described above.

Because there were 80 pots in each of three replicates, it was difficult to eliminate variation using a randomised block design and therefore the pots were ordered in four cyclic designs within each of the three replicates (John, Wolcock and David, 1972). Because the replicates were split into four incomplete blocks, some of the interactions were confounded in a balanced way. The design was provided by E.R. Williams and R. Thompson, Unit of Statistics, University of Edinburgh.

The experiment was carried out on the three soils at different times as it was logistically impossible to run the experiment concurrently. The experiment with the brown earth was sown on 5 March 1975 and was first harvested 10 weeks later on 12 May 1975 with the second harvest on 10 June 1975, 14 weeks after sowing. The corresponding dates for the experiment in the peat soil were 20 March 1975, 27 May 1975 and 24 June 1975 and, for the dry peat, 26 May 1975, 2 August 1975 and 30 August 1975. There were the same time intervals between harvests.

Chemical analysis of shoots was carried out on plants which received phosphorus and potassium treatments with nil and 80 kg N/ha in the brown earth and deep peat. The dry matter yields and nutrient contents of shoots, for each treatment, at each harvest, and in each soil, are given in Appendices 2 and 3. Statistical analysis, which took the cyclic design into account, was only possible for yield. Other analysis (with missing data for 20 and 40 kg N/ha) was based upon randomised blocks.

RESULTS

Yield (a) Growth in the soils (Table 5)

Because plants were grown in each soil at different times of the year, it was not possible to compare absolute levels of yield between soils so emphasis was placed on relativities. The maximum yields from each soil reflected the time of year that the experiment was carried out: dry peat > deep peat > brown earth. The summer of 1975 was very warm and plants in the Glensaugh dry peat grew rapidly until the first harvest (the growth period was set when the first harvest was taken from the brown earth) where the maximum dry matter yield of shoots was 14.4 g/pot, which is approximately equivalent to 20,000 kg/ha. Thereafter there was much less growth.

The minimum yields were ordered differently from the maximum: brown earth > dry peat > deep peat, and reflected more the order expected from soil analysis (see Table 3).

Table 5: The yield (DM g/pot) from white clover grown in three hill soils at different times of the year (Experiment 1)

Soil	Harvest date	Dry matter yield of shoot (g/pot)	
		mean	range
Sourhope Brown Earth	1 12/5/75	2.7	1.4 - 3.8
	2 10/6/75	4.9	2.6 - 7.7
	1+2	7.6	4.0 - 11.5
Glensaugh Dry Peat	1 2/8/75	6.7	0.8 - 14.4
	2 30/8/75	2.6	1.0 - 4.9
	1+2	9.3	1.8 - 19.3
Lephinmore Deep Peat	1 27/5/75	2.2	0.1 - 6.1
	2 24/6/75	2.7	0.5 - 9.8
		4.9	0.6 - 15.9

(b) Main effects from added nitrogen, phosphorus and potassium (Table 6)

Added nitrogen (0-80 kg N/ha) had no effect on yield on any soil at either harvest, except in the dry peat where the total growth (H1 + 2) was slightly depressed by all levels of added nitrogen.

There was a response in all soils at both harvests to phosphorus. The response was small in the brown earth (H1 + 2, 0.3 fold*) relative to the peats (H1 + 2, 2.6 fold, dry peat: 6.3 fold, deep peat).

The response to potassium was of a similar magnitude to that with phosphorus. The clover in the brown earth responded relatively less (H1 + 2, 0.4 fold) than in the peats (H1 + 2, 1.8 fold, dry peat: 3.6 fold, deep peat). In the brown earth, at the first harvest there was a

* a fold increase in dry weight was calculated as follows:

$$\frac{\text{fertilized yield - unfertilized yield}}{\text{unfertilised yield}}$$

Table 6. The main effects of nitrogen, phosphorus and potassium fertilizers on the shoot dry weight of white clover (g/pot) when grown in three soils in pot experiments (Experiment 1)

Soil	Nutrient	Harvest	Level of nutrient (kg/ha) 0	20	40	80	160	320	SED (78 df)
Sourhope brown earth	Nitrogen	1	2.7	2.7	2.7	2.7	-	-	0.09
		2	5.0	4.8	4.9	4.8	-	-	0.15
		1+2	7.7	7.5	7.6	7.5	-	-	0.20
	Phosphorus	1	2.2	-	2.6	2.9	3.3	-	0.09
		2	4.4	-	4.9	5.2	5.1	-	0.15
		1+2	6.6	-	7.5	8.1	8.4	-	0.20
	Potassium	1	2.7	-	2.8	2.8	2.8	2.4	0.10
		2	3.4	-	4.1	5.0	5.5	6.3	0.17
		1+2	6.1	-	6.9	7.8	8.3	8.7	0.22
Glensaugh dry peat	Nitrogen	1	6.9	6.7	6.6	6.6	-	-	0.21
		2	2.7	2.5	2.5	2.5	-	-	0.08
		1+2	9.6	9.2	9.1	9.1	-	-	0.21
	Phosphorus	1	2.0	-	7.3	8.6	8.9	-	0.21
		2	1.3	-	3.1	3.0	2.9	-	0.08
		1+2	3.3	-	10.4	11.6	11.8	-	0.21
	Potassium	1	3.2	-	5.1	6.5	9.0	9.7	0.23
		2	1.4	-	2.4	2.6	3.0	3.4	0.09
		1+2	4.6	-	7.5	9.1	12.0	13.1	0.24
Lephinmore deep peat	Nitrogen	1	2.1	2.1	2.2	2.2	-	-	0.07
		2	2.7	2.5	2.7	2.7	-	-	0.12
		1+2	4.8	4.6	4.9	4.9	-	-	0.14
	Phosphorus	1	0.3	-	1.6	2.9	3.9	-	0.07
		2	0.9	-	1.8	3.1	4.9	-	0.12
		1+2	1.2	-	3.4	6.0	8.8	-	0.14
	Potassium	1	0.6	-	2.1	2.6	2.7	2.8	0.08
		2	0.9	-	2.0	2.8	3.3	4.1	0.14
		1+2	1.5	-	4.2	5.4	6.0	6.9	0.16

depression in yield with the greatest level of added potassium (320 kg K/ha).

Photographs of the clover growing in the Lephinmore peat just before the first harvest show the response to phosphorus when potassium was in adequate supply (Fig. 3b) and the response to potassium when phosphorus was in adequate supply (Fig. 3c).

(c) Growth interactions between added nitrogen, phosphorus and potassium (Figs. 4, 5 and 6)

There were no interactions between nitrogen and either phosphorus or potassium.

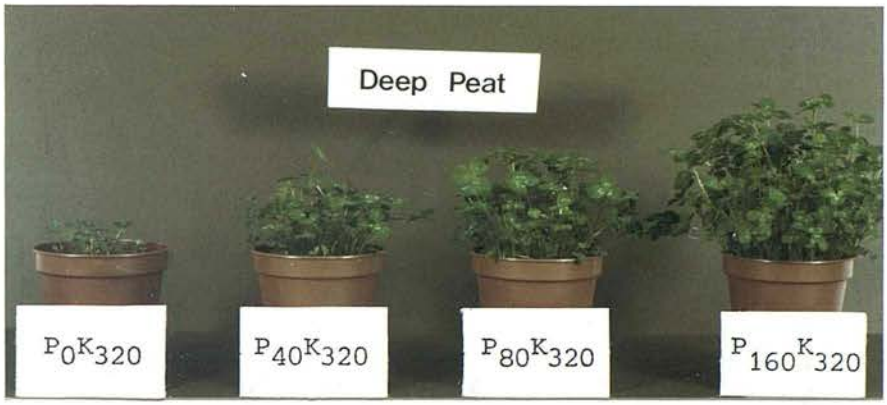
There was a marked growth interaction by white clover when phosphorus and potassium fertilizers were applied together to both of the peats and also to a lesser extent to the brown earth.

The yield when phosphorus and potassium fertilizers were applied together was greater than the additive yield of each fertilizer applied in the absence of the other: the interaction was synergistic. The shoot dry matter yields (g/pot) of white clover when given different combinations of phosphorus and potassium fertilizers are illustrated for the brown earth in Fig. 4, for the dry peat in Fig. 5, and for the deep peat in Fig. 6.

The surface of Fig. 4a (brown earth, harvest 1) is undulating with some synergism from 0-80 kg P/ha and 0-80 kg K/ha. The surface in Fig. 4b (brown earth, harvest 2) is steeper with the interaction occurring from 0-80 kg P/ha to 0-160 kg K/ha.

(a)

(b)



(c)

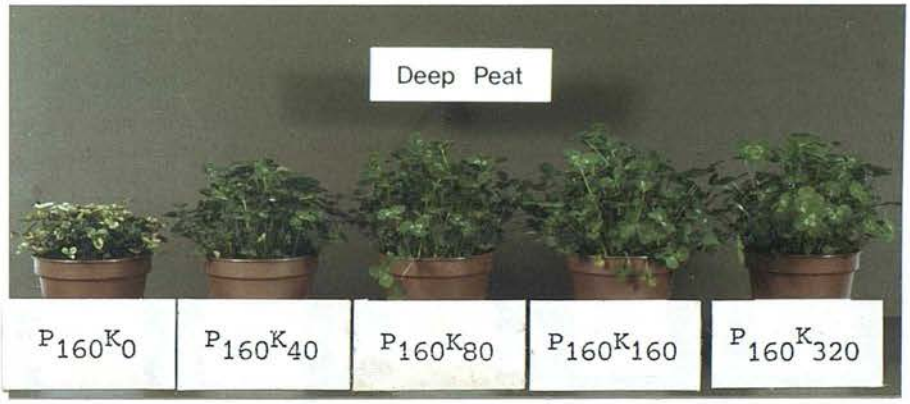


Figure 3. The responses by white clover to potassium and phosphorus fertilizers grown in deep peat soil
(a) The response to phosphorus in the absence of potassium
(b) The response to phosphorus in the presence of potassium (320 kg K/ha)
(c) The response to potassium in the presence of phosphorus (160 kg P/ha)
(Experiment 1)

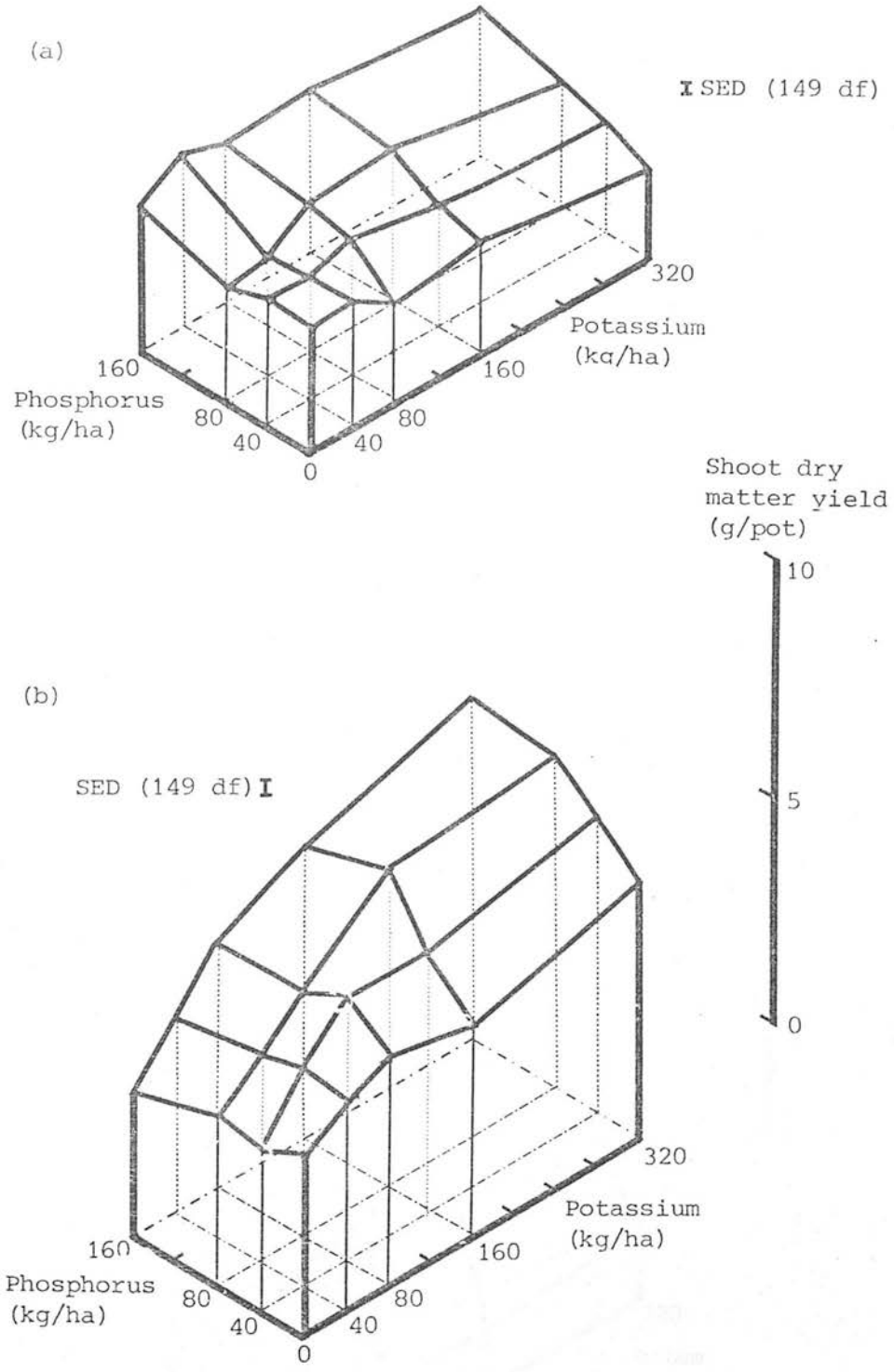


Figure 4. The responses (shoot dry matter, g/pot) from white clover given phosphorus and potassium fertilizers when grown in Sourhope brown earth (a) Harvest 1 (b) Harvest 2 (Experiment 1).

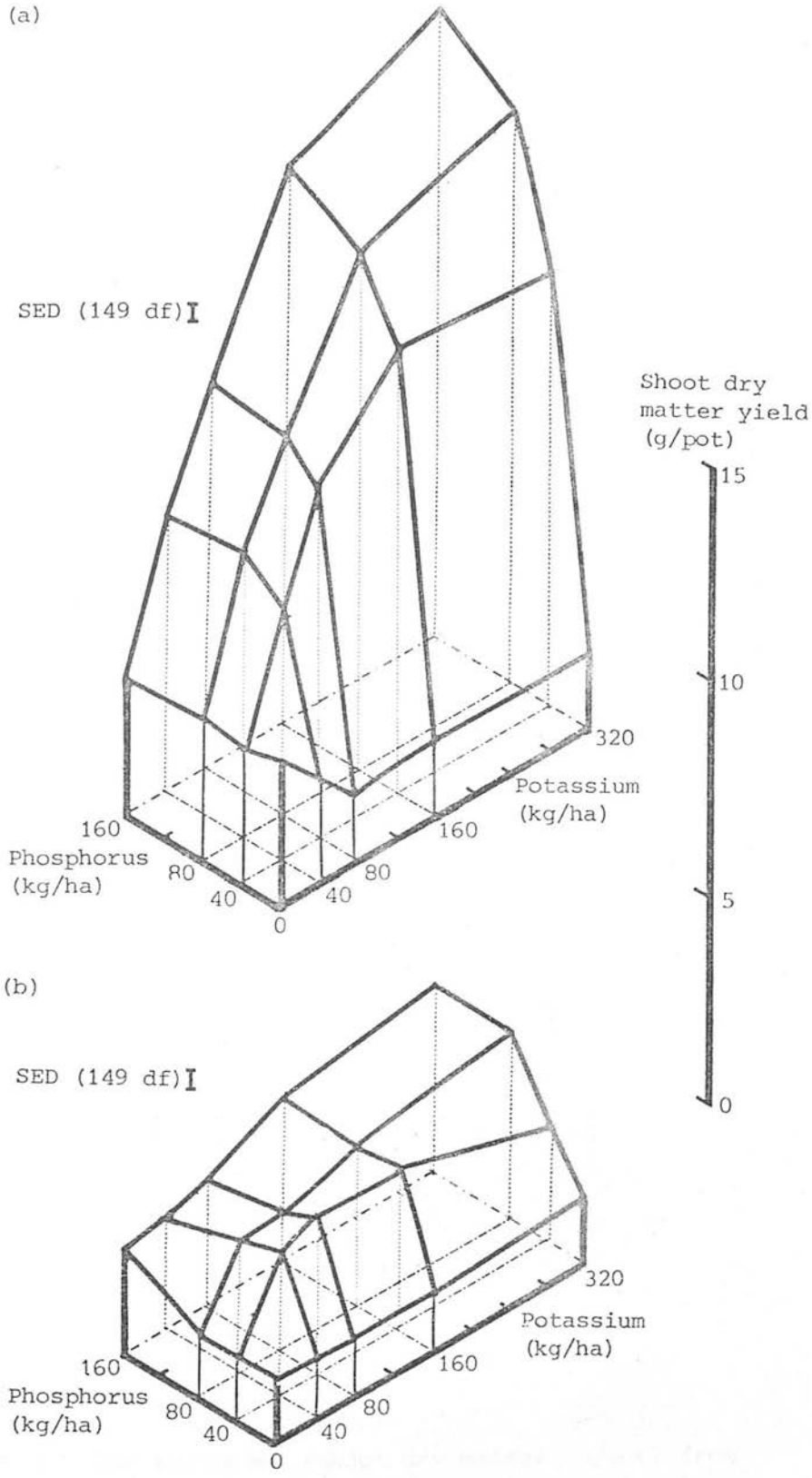


Figure 5. The responses (shoot dry matter, g/pot) from white clover given phosphorus and potassium fertilizers when grown in Glensaugh dry peat (a) Harvest 1 (b) Harvest 2 (Experiment 1)

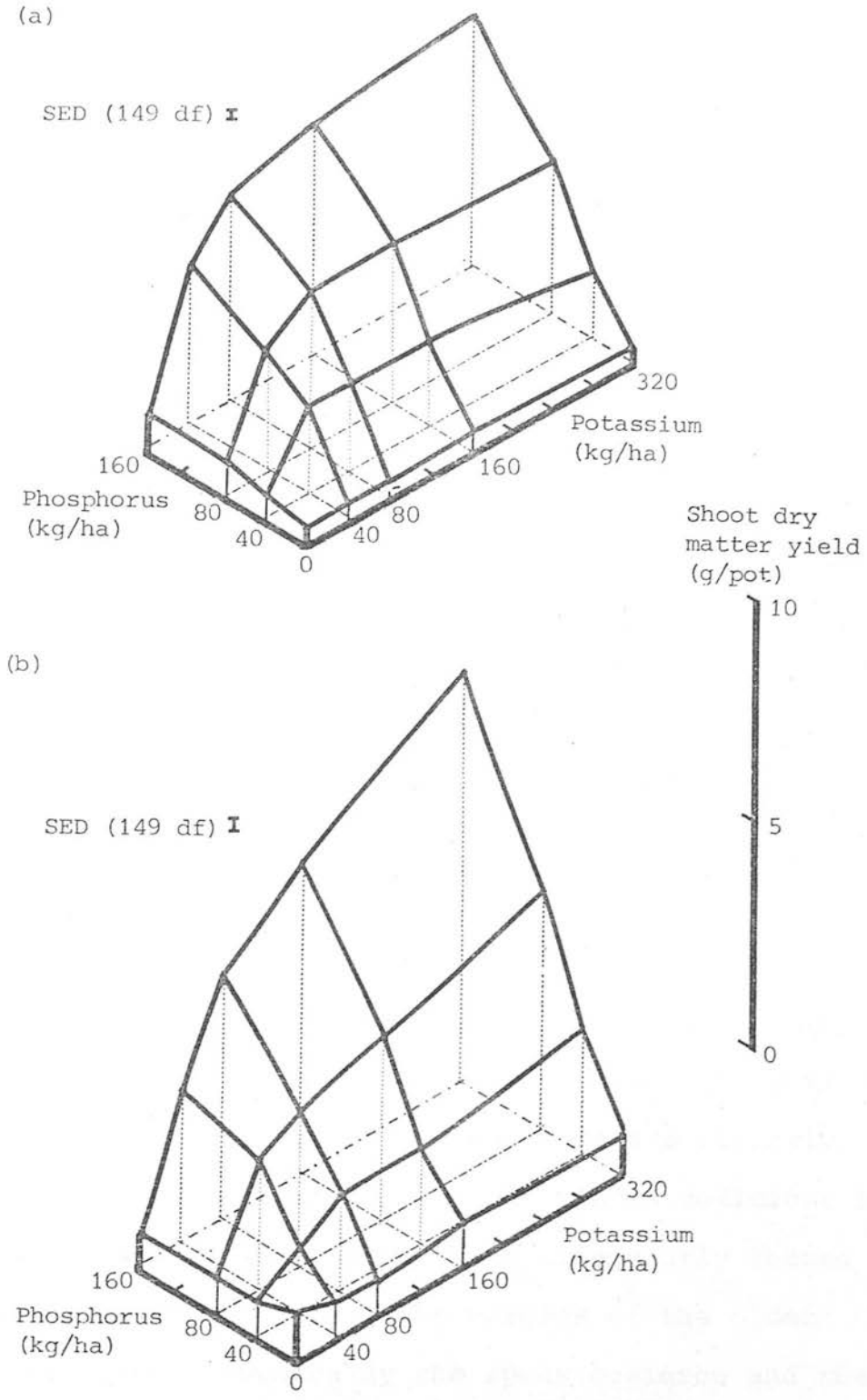


Figure 6. The responses (shoot dry matter, g/pot) from white clover given phosphorus and potassium fertilizers when grown in Lephinmore deep peat (a) Harvest 1 (b) Harvest 2 (Experiment 1)

The interaction in the dry peat at harvest 1 (Fig. 5a) occurred from 0-80 kg P/ha and 0-160 kg K/ha and there was a large response to fertilizer. At harvest 2 (Fig. 5b), there was little response to the fertilizer treatment but there was some interaction from 0-40 kg P/ha with 0-40 kg K/ha.

In the Lephinmore peat (Fig. 6) there was a large response to fertilizer at both harvests and interaction occurred with 0-80 kg P/ha and 0-80 kg K/ha at harvest 1 and with 0-160 kg P/ha and 0-320 kg K/ha at harvest 2. Figs. 3a and 3b are photographs which show clover growing in the Lephinmore peat just before the first harvest, where there is little response to phosphorus in the absence of potassium and a large response in the presence of 320 kg K/ha.

Deficiency symptoms

Plants deficient and sufficient in phosphorus are shown in Fig. 7. The deficient plants are spindly with small leaves and no stolons and the stems are slightly red (see also Figs. 3a and 3b). The plants deficient in potassium are very distinctive, with irregularly shaped necrotic spots which form on the margins of the older leaves (Fig. 8). Eventually the spots coalesce and the leaf dies. The growth of plants in soils with a low level of phosphorus is slow relative to phosphorus sufficient plants but the growth of plants with a low level of potassium is similar to those with a greater amount until just before deficiency symptoms begin to

a)

b)

Figure 7. White clover plants a) sufficient in phosphorus; b) deficient in phosphorus

Figure 8. White clover plants deficient in potassium

appear on the leaves.

None of the plants had nitrogen deficiency symptoms.

The concentration of nitrogen, phosphorus and potassium in the shoots in relation to yield

(a) Nitrogen (Table 7)

Chemical analysis of shoots was done on the two extreme nitrogen treatments, nil and 80 kg N/ha. These treatments had no effect on yield, nor on the concentration of nitrogen in the shoots of clover grown in the Sourhope soil. However, at the second harvest in the Lephinmore peat the concentration of nitrogen in the shoots of the plants which received 80 kg N/ha (2.27% N) was less than those without added nitrogen (2.68%). The differences in nitrogen concentrations were reflected in the nitrogen content of the shoots.

Table 7: The effect of two nitrogen treatments (nil and 80 kg N/ha) on the dry matter yield of shoots, the concentration of nitrogen and the amount of nitrogen in the shoots of white clover grown in two soils in a pot experiment and harvested twice (Experiment 1)

Soil	Harvest	Yield (g/pot) of shoot		%N		N in (mg) shoot	
		NO	N80	NO	N80	NO	N80†
Sourhope Brown earth	H1	2.7	2.7	3.80	3.84	101	103
	H2	5.0	4.8	2.97	3.14	148	151
	H1+H2	7.7	7.5	3.25	3.38	249	254
Lephinmore Deep peat	H1	2.1	2.2	2.90	3.10	61	68
	H2	2.7	2.7	2.68	2.27	72	60
	H1+H2	4.8	4.9	2.78	2.64	133	128
SED (78 df) ††		0.10		0.04		3	

† 56 mg N applied.

†† Data for the two soils were analysed separately but each set had the same value for standard error of difference between means.

The treatment 80 kg N/ha supplied 56 mg N/pot. In both soils plants had absorbed this amount of nitrogen during the first growth period. Assuming all the applied nitrogen was absorbed, 198 mg N in the brown earth and 72 mg N in the deep peat came from either the soil or from biological fixation. The amount of nitrogen mineralised from the soil in the experiment under glasshouse conditions is unknown and is difficult to estimate but some of the extra nitrogen probably came from biological fixation. Haystead and Marriott (1979a) measured mineralisation from the Lephinmore peat in a pot experiment and calculations from their data suggest that 80 mg N per pot may be mineralised over a 12 week period. This could be sufficient to supply the additional need of the plants without the need for biologically fixed nitrogen. No similar data are available for the Sourhope brown earth.

(b) Phosphorus and potassium (Tables 8 and 9)

The concentration above which a nutrient is non-limiting to growth corresponds to the concentration above which there is no response in yield. Investigation of the concentrations of phosphorus and potassium in the shoots of clover grown in both soils at both harvests indicates that the response to potassium declined when the concentration in the shoot reached 0.7-0.9% K. Harvest 2 of the experiment in the brown earth soil and both harvests of the experiment in the peat suggested that there was little response to phosphorus when the concentration was greater than 0.18-0.20% P. However, small but

Table 8. The dry matter yields of the shoot (g/pot) and phosphorus concentrations in the shoot of white clover given combinations of phosphorus and potassium fertilizers and sampled on two occasions (Experiment 1)

Soil	Harvest		P0		P40		P80		P160	
			Yield	%P	Yield	%P	Yield	%P	Yield	%P
Sourhope brown earth	1	K0	2.5	0.29	2.7	0.30	2.6	0.32	2.7	0.36
		K40	2.0	0.23	2.5	0.27	2.8	0.33	3.6	0.34
		K80	1.7	0.29	2.7	0.28	3.0	0.34	3.5	0.32
		K160	2.5	0.24	2.8	0.27	3.0	0.30	3.5	0.34
		K320	2.0	0.25	2.2	0.28	2.6	0.30	2.8	0.36
			SED (78 df)		Yield = 0.21		%P = 0.008			
	2	K0	3.9	0.24	3.2	0.28	3.8	0.33	3.2	0.45
		K40	4.4	0.18	4.3	0.21	4.4	0.29	3.9	0.41
		K80	4.3	0.17	5.4	0.21	5.3	0.24	5.0	0.35
		K160	4.3	0.17	5.5	0.20	6.4	0.20	6.5	0.31
		K320	5.5	0.15	5.9	0.17	6.9	0.19	7.2	0.26
			SED (78 df)		Yield = 0.35		%P = 0.010			
Lephinmore peat	1	K0	0.2	0.11	0.5	0.18	0.7	0.28	0.8	0.39
		K40	0.3	0.10	2.2	0.09	2.9	0.13	3.5	0.22
		K80	0.3	0.10	2.1	0.09	3.6	0.11	4.3	0.20
		K160	0.2	0.11	1.9	0.09	3.7	0.10	4.9	0.19
		K320	0.4	0.11	1.7	0.09	3.5	0.11	5.8	0.16
			SED (78 df)		Yield = 0.20		%P = 0.006			
	2	K0	0.1	0.11	0.9	0.22	0.9	0.31	0.9	0.44
		K40	0.8	0.08	1.6	0.12	2.7	0.18	3.0	0.28
		K80	0.8	0.09	2.0	0.10	3.5	0.17	5.3	0.29
		K160	1.0	0.09	1.9	0.09	3.7	0.14	5.8	0.28
		K320	0.9	0.09	3.0	0.09	5.3	0.15	9.3	0.24
			SED (78 df)		Yield = 0.23		%P = 0.013			

Table 9. The dry matter yields of the shoot (g/pot) and the potassium concentrations in the shoot of white clover given combinations of phosphorus and potassium fertilizers and sampled on two occasions (Experiment 1)

Soil	Harvest		P0		P40		P80		P160	
			Yield	%K	Yield	%K	Yield	%K	Yield	%K
Sourhope brown earth	1	K0	2.5	1.54	2.7	1.36	2.6	1.37	2.7	1.08
		K40	2.0	1.93	2.5	1.87	2.8	1.95	3.6	1.56
		K80	1.7	2.80	2.7	2.23	3.0	2.13	3.5	1.95
		K160	2.5	3.29	2.8	3.04	3.0	2.86	3.5	2.74
		K320	2.0	4.04	2.2	4.01	2.6	3.95	2.8	4.00
			SED (78 df)		Yield = 0.21		%K = 0.054			
	2	K0	3.9	0.61D†	3.2	0.55D	3.8	0.56D	3.2	0.53D
		K40	4.3	0.71	4.3	0.73D	4.4	0.68D	3.9	0.64D
		K80	4.3	1.00	5.4	0.78	5.3	0.74	5.0	0.63D
		K160	4.3	1.43	5.5	1.00	6.4	0.87	6.5	0.85
		K320	5.5	2.38	5.9	2.23	6.9	1.56	7.2	1.57
			SED (78 df)		Yield = 0.35		%K = 0.068			
Lephinmore peat	1	K0	0.2	0.67	0.5	0.32	0.7	0.30	0.8	0.32
		K40	0.3	2.27	2.2	0.76	2.9	0.54	3.5	0.44
		K80	0.3	3.28	2.1	1.38	3.6	0.82	4.3	0.58
		K160	0.2	3.43	1.9	2.69	3.7	1.61	4.9	0.85
		K320	0.4	3.78	1.7	3.08	3.5	2.93	5.8	2.12
			SED (78 df)		Yield = 0.20		%K = 0.095			
	2	K0	0.1	0.46D	0.9	0.33D	0.9	0.29D	0.9	0.29D
		K40	0.8	1.52	1.6	0.54D	2.7	0.41D	3.0	0.39D
		K80	0.8	2.39	2.0	0.84	3.5	0.51D	5.3	0.42D
		K160	1.0	2.78	1.9	1.62	3.7	0.79	5.8	0.48D
		K320	0.9	3.15	3.0	2.53	5.3	1.22	9.3	0.74
			SED (78 df)		Yield = 0.23		%K = 0.064			

† D = plants with potassium deficiency symptoms recorded at harvest 2

significant responses to phosphorus occurred in the Sourhope soil at harvest 1 when the concentration of phosphorus in the shoot was greater than 0.3% P.

The depression in yield of white clover with 320 kg K/ha at the first harvest in the Sourhope brown earth was associated with a concentration of potassium in the shoots of 4.0%.

The concentrations of phosphorus and potassium in the shoots of clover grown in the deep peat and the brown earth at both harvests are plotted against each other in Fig. 9. If, from the information above, the concentration of phosphorus below which deficiency may occur is taken as 0.20% and the concentration of potassium below which deficiency may occur is taken as 0.9%, then all plants grown in the peat were either deficient in phosphorus or potassium, as were all plants grown in the brown earth at harvest 2. Clover grown in the brown earth until harvest 1 was sufficient in both phosphorus and potassium.

The plants with potassium deficiency symptoms were noted on both soils at harvest 2 (Table 9) and they occurred on plants with less than 0.8% K in the shoot.

The effect of added nitrogen, phosphorus and potassium on the concentration of calcium and magnesium in the shoot

(a) Nitrogen (Table 10)

The nitrogen fertilizer in the experiment was supplied in the form of nitrate ($Ca(NO_3)_2$). According

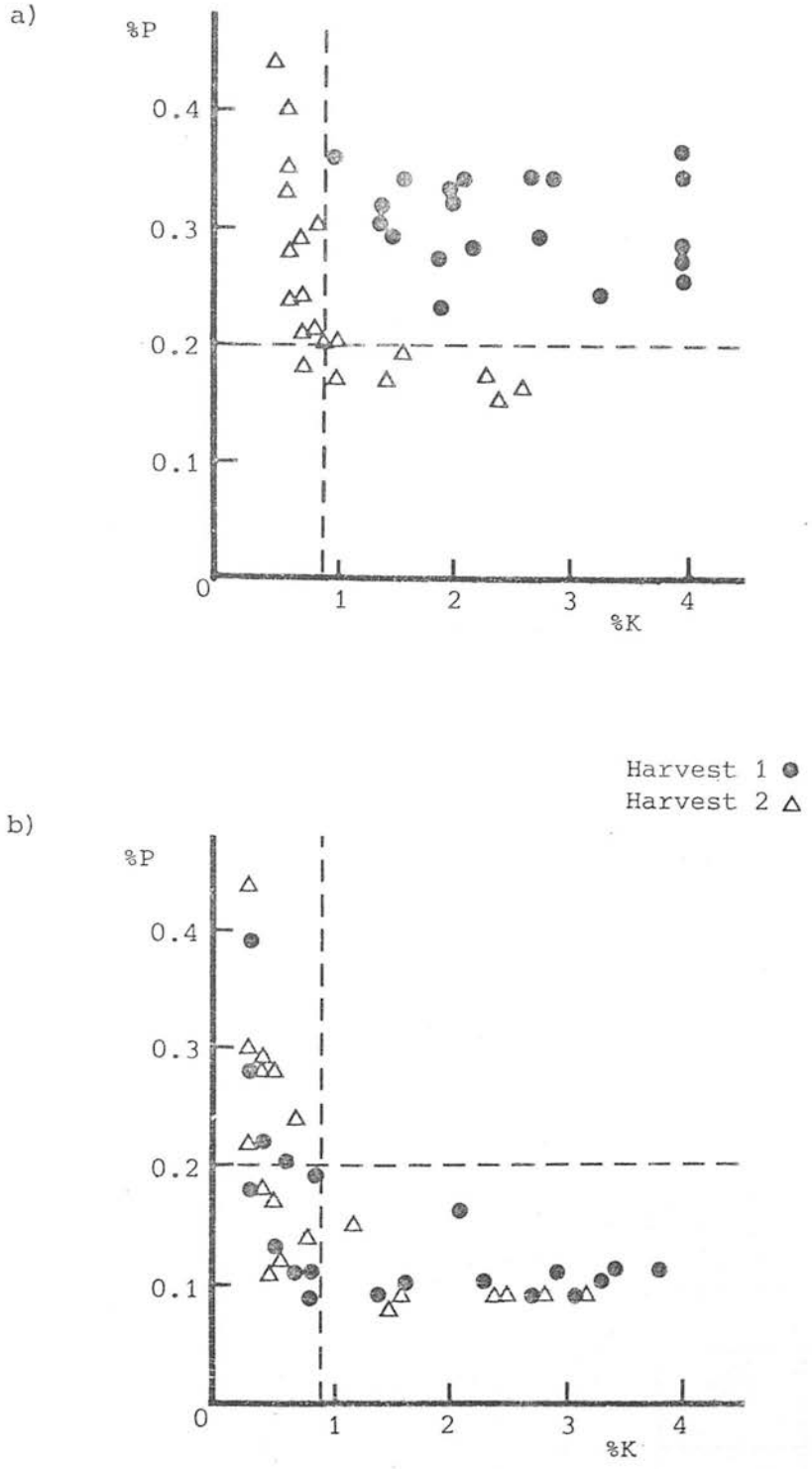


Figure 9. The concentrations of phosphorus and potassium in the shoots of white clover given phosphorus and potassium fertilizers and grown in two soils (a) Sourhope brown earth (b) Lephinmore deep peat (Experiment 1).
The broken lines indicate the concentrations of nutrient in the shoot below which deficiency may occur.

to Raven and Smith (1976) the by-products from nitrate assimilation are organic anions which are neutralised by inorganic cations and the cation/organic anions are stored in the cell vacuole. The net product from biological nitrogen fixation are H^+ ions which are excreted into the soil (see p.21). Therefore the uptake of cations will be greater in plants which derive most of their nitrogen from nitrate than in those which derive their nitrogen from biological fixation. In the experiment there was sufficient nitrogen added for at least half the growth in the brown earth and almost all the growth in the peat at harvest 1 when plants were given 80 kg N/ha as nitrate (see Table 7). It was therefore possible that the cation contents of the shoots of plants given 80 kg N/ha at harvest 1 may have been greater than those without nitrogen fertilizer. There was no effect in the Sourhope brown earth, but in the Lephinmore peat there was more potassium and less magnesium in plants given nitrogen fertilizer.

Table 10: The effect of two nitrogen treatments (nil and 80 kg N/ha) on cation concentrations in the shoots of white clover (Experiment 1)

Soil	Harvest	Nutrient	NO	N80	SED (78 df)
Sourhope Brown Earth	1	%K	2.49	2.40	0.045
		%Ca	2.65	2.65	0.038
		%Mg	0.63	0.61	0.013
Lephinmore Deep Peat	1	%K	1.54	1.68	0.030
		%Ca	2.22	2.25	0.043
		%Mg	0.53	0.48	0.012

(b) Phosphorus (Fig. 10)
The concentrations of calcium and magnesium in plants grown in the Sourhope soil ranged from 1.8-3.5% and 0.4-1.0%

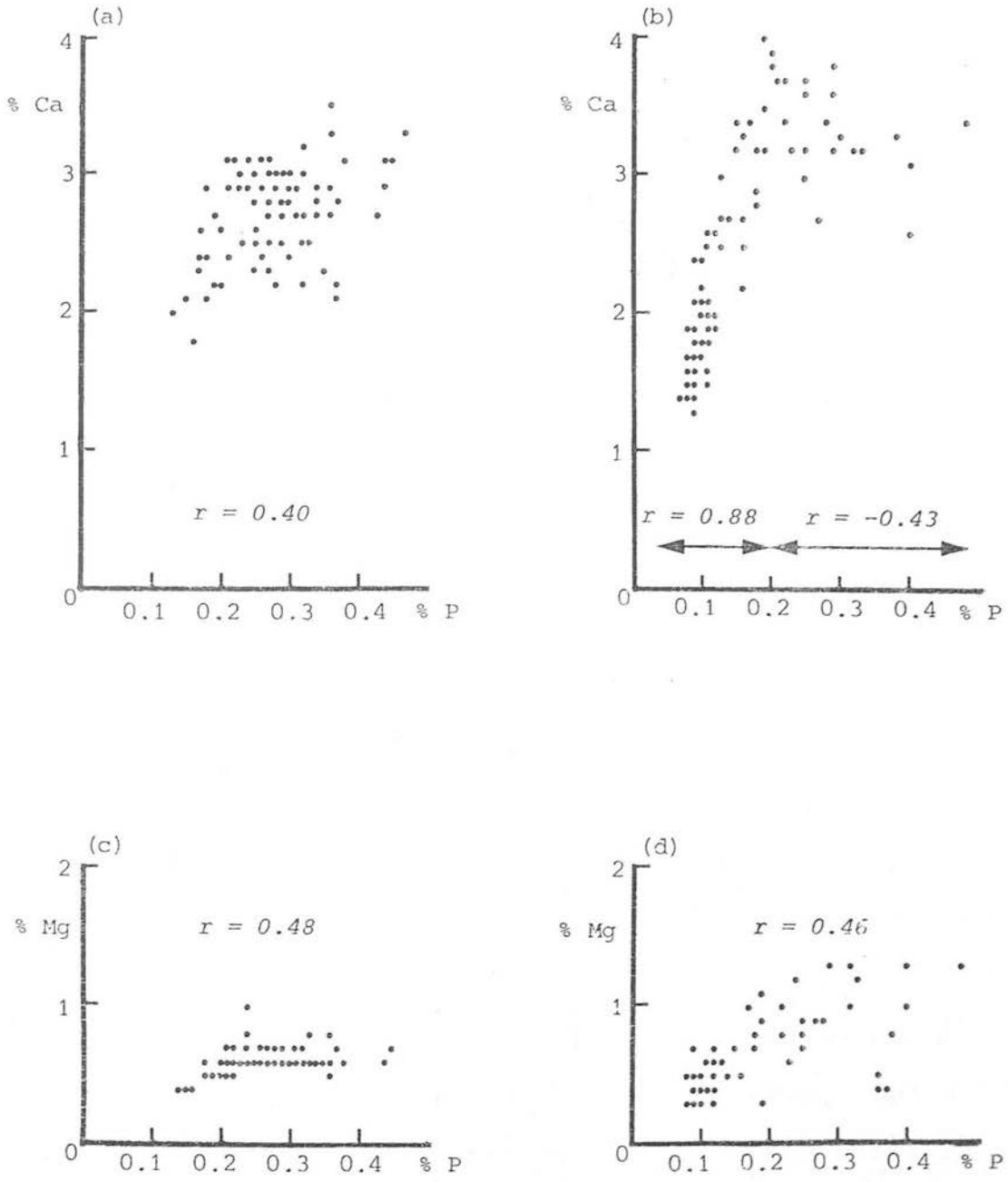


Figure 10. The effect of increasing the concentration of phosphorus in the shoot on the concentrations of calcium and magnesium in the shoots of white clover grown in Sourhope brown earth, (a) and (c) and the Lephinmore deep peat, (b) and (d) (Experiment 1).

respectively. The concentrations of calcium and magnesium in plants grown in the Lephinmore soil had a wider range from 1.2-4.0% and 0.3-1.3% respectively.

Where the concentration of phosphorus was less than 0.2% there seemed to be a relationship between it and the calcium content of the plant, particularly when grown in the peat (Fig. 10b). Since the range of phosphorus concentrations in the shoot was the result of fertilizer treatment (see Table 8), phosphorus fertilization which increases the concentration of phosphorus in the shoot from less than .2% to .2% or more can also increase the concentration of calcium.

There was little relationship between the concentration of phosphorus and magnesium in the plant shoots.

(c) Potassium (Fig. 11)

There was a greater relationship between the concentration of potassium in the shoots and the concentration of calcium and magnesium in the shoots of plants grown in the Lephinmore peat than in the Sourhope brown earth. Since the range of potassium levels in the shoots was a result of fertilizer treatment (Table 9), added potassium which increased the concentration of potassium from 0.3 to 2.0% can also decrease the concentration of calcium. Increases in the concentration of potassium from 0.3 to 1.0% decreased the magnesium content of plants grown in the peat.

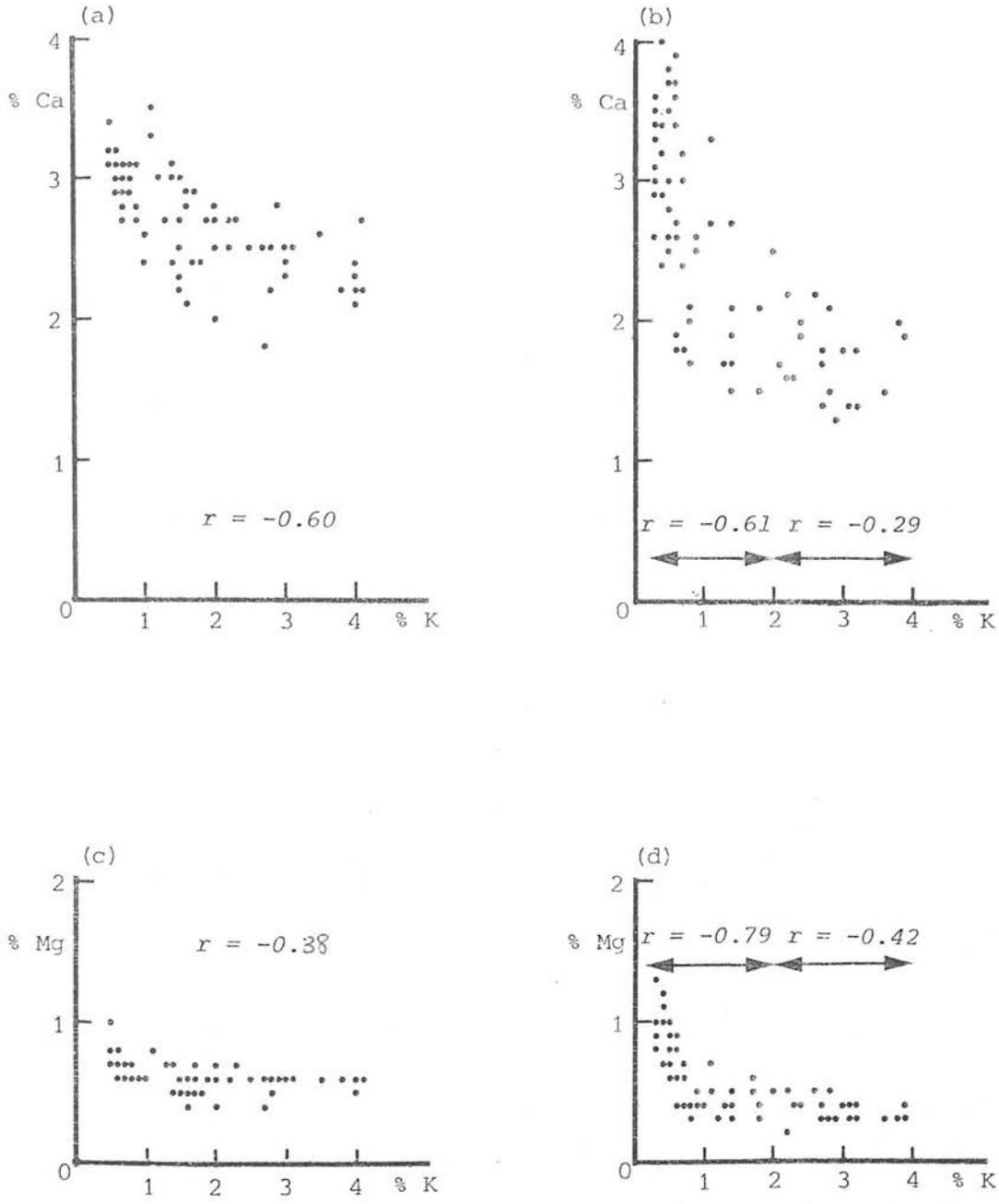


Figure 11. The effect of increasing the concentration of potassium in the shoot on the concentrations of calcium and magnesium in the shoots of white clover grown in Sourhope brown earth, (a) and (c) and the Lephinmore deep peat, (b) and (d) (Experiment 1).

The effect of phosphorus and potassium on the nitrogen content of the shoot

There was no count of the number of root nodules, nor was there a measure of the rate of nitrogen fixation. The only estimate of nitrogen fixation in the experiment was the nitrogen content of the shoots of plants which did not receive nitrogen fertilizer. Some of this nitrogen would come from the soil and, from the information of Haystead and Marriott (1979a), this could have been up to 80 mg N/pot in 12 weeks in the Lephinmore peat and may have been more in the Sourhope brown earth.

(a) Phosphorus (Table 11)

The concentration of nitrogen in the shoots increased as the level of added phosphorus increased from 40-160 kg P/ha, despite the fact that there was a response in dry matter. An increase in yield would reduce the concentration of nitrogen in the shoots unless there was a corresponding increase in fixation.

(b) Potassium (Table 12)

The concentration of nitrogen in the shoots remained similar in both soils and at both harvests as the level of potassium fertilizer was increased from 40-320 kg K/ha.

Nutrient uptake from unfertilized soils

(a) Phosphorus (Table 13)

The uptake of phosphorus from the brown earth was 6 mg/100 g soil and, from the deep peat, 2 mg/100 g soil. This was the same as the phosphorus measured as available by the ammonium acetate extractant in the brown earth and

Table 11: The effect of added phosphorus on the concentration of nitrogen in the shoots of white clover when no nitrogen was added (Experiment 1)

Soil	Harvest	% Nitrogen in shoot				SED
		PO	P40	P80	P160	(78 df)
Sourhope Brown Earth	1	3.9	3.6	3.6	4.1	0.11
	2	2.8	2.9	2.8	3.4	0.11
Lephinmore Deep Peat	1	2.9	2.6	2.7	3.4	0.09
	2	2.1	2.3	2.8	3.5	0.05

Table 12: The effect of added potassium on the concentration of nitrogen in the shoots of white clover when no nitrogen was added (Experiment 1)

Soil	Harvest	%Nitrogen in shoot					SED
		KO	K40	K80	K160	K320	(78 df)
Sourhope Brown Earth	1	3.8	3.8	3.7	3.7	3.9	0.12
	2	3.1	3.0	3.0	2.7	3.0	0.12
Lephinmore Deep Peat	1	3.8	2.7	2.6	2.6	2.8	0.10
	2	3.6	2.6	2.5	2.4	2.3	0.06

Table 13: The uptake of phosphorus, when no phosphorus fertilizer was applied and potassium when no potassium fertilizer was applied, by white clover from two soils in a pot experiment (Experiment 1)

Uptake	Harvest	Phosphorus			Potassium		
		Brown Earth	Peat	Ratio	Brown Earth	Peat	Ratio
mg/pot†	1 + 2	14	1	14.0	54	5	10.8
mg/100g soil	1 + 2	6	2	3.5	23	6	3.7
mg/l soil	1 + 2	38	2	19.0	152	6	25.3

† Approximately 240 g dry brown earth and 75 g peat per pot

more than that measured in the deep peat (see Table 3) of 6 and 1 mg P/100 respectively. The unfertilized brown earth yielded 3.5 times as much phosphorus as the deep peat on the basis of equal weights and 19 times as much phosphorus on the basis of equal volumes of soil.

(b) Potassium (Table 13)

The amount of available potassium extracted from the soil using ammonium acetate was 75 and 15 mg K/100 g soil, for the brown earth and deep peat respectively. This was probably an overestimate because 23 and 6 mg K/100 g soil were taken up in the shoots of white clover although the amount in the root was not measured.

The brown earth yielded 3.7 times as much potassium as the peat when calculated on the basis of the weight of soil but the brown earth provided 25 times more potassium than the peat on the basis of volume.

Fertilizer uptake from the soils

The amount of fertilizer recovered in the shoots was calculated as follows:-

$$\frac{\text{nutrient uptake when fertilized} - \text{nutrient uptake when not fertilized}}{\text{amount of nutrient applied}} \times 100$$

(a) Phosphorus (Table 14)

The overall uptake of phosphorus fertilizer was the same in both soils (14%) even though the brown earth contained more indigenous available phosphorus. In the brown earth most of the phosphorus was taken up at intermediate levels of potassium but, in the deep peat, the

percentage of phosphorus fertilizer taken up increased as the level of both added phosphorus and potassium increased.

(b) Potassium (Table 15)

A large proportion of the potassium fertilizer was recovered in both soils and, overall, there was a 77% recovery in the brown earth and 64% recovery in the peat. In general, the amount recovered in both soils increased with the level of added phosphorus.

The following experiment examines how phosphorus and potassium affect growth of leaves of white clover and why the effect appears to be synergistic.

EXPERIMENT 2. The effect of phosphorus and potassium fertilizer on leaf growth

EXPERIMENTAL

The data presented here are from the second harvest of a pot experiment designed to measure the long term effects on growth of inoculation with mycorrhizal fungi when white clover was given different levels of phosphorus and potassium fertilizer and grown with mineral nitrogen or biologically fixed nitrogen (see Part II, Experiment 9). The growth interaction between phosphorus and potassium was most apparent between the first and second harvests of the experiment which was harvested eight times.

The fertilizer treatments were:-

No phosphorus or potassium fertilizer			(POKO)
40 kg P/ha	:	40 kg K/ha	(P40K40)
40 kg P/ha	:	160 kg K/ha	(P40K160)

Table 14. The percentage of phosphorus fertilizer recovered in the shoots of white clover after two harvests when given phosphorus and potassium fertilizers in a pot experiment (Experiment 1)

Soil		Percentage phosphorus fertilizer recovered				SED (58 df)
		P40	P80	P160	Mean	
Sourhope Brown Earth	KO	4	7	7	6	
	K40	11	16	14	14	
	K80	25	20	14	20	
	K160	14	16	16	15	
	K320	11	14	14	13	
	Mean	15	15	13	14	1.7
Lephinmore Deep Peat	KO	7	8	5	7	
	K40	9	13	13	12	
	K80	9	16	21	16	
	K160	11	15	22	16	
	K320	7	19	28	18	
	Mean	9	14	17	14	1.0

Table 15. The percentage of potassium fertilizer recovered in the shoots of white clover after two harvests when given phosphorus and potassium fertilizers in a pot experiment (Experiment 1)

Soil		Percentage potassium fertilizer recovered					SED (62 df)
		PO	P40	P80	P160	Mean	
Sourhope Brown Earth	K40	31	84	97	100	78	
	K80	51	86	84	94	79	
	K160	71	71	77	92	78	
	K320	67	72	70	79	73	
	Mean	55	79	82	91	77	11.5
Lephinmore Deep Peat	K40	57	70	77	79	72	
	K80	43	71	78	75	67	
	K160	28	71	77	61	59	
	K320	15	51	75	85	57	
	Mean	36	66	77	75	64	4.1

160 kg P/ha : 40 kg K/ha (P160K40)

160 kg P/ha : 160 kg K/ha (P160K160)

In addition, basal dressings of 2.7 tonnes $CaCO_3$/ha and 10 kg Mg/ha (as $MgSO_4.7H_2O$) were mixed into the Lephinmore peat. Trace elements were watered onto the plants throughout the experiment using the recipe from the Dart and Pate nutrient solution (Dart and Pate, 1959). All plants were inoculated with a suspension of rhizobia one week after germination.

The experiment was housed in a growth room (see p. 46 for the experimental conditions). Twenty seeds were sown on 19 January 1979 in 7.5 cm plant pots and thinned to ten healthy specimens per pot after germination. Assessments made which are used in this section are:-

(a) the total number and the number of green leaves.

(b) the length of the central leaflet from 20 green leaves (when sufficient).

(c) the leaf area of 20 green leaves. The area of leaves with central leaflets from 3-17 mm long was measured by passing ten leaves of each length through a leaf area meter designed by A.R.M. Chambers, HFRO. The table of leaf length with leaf area is given in Appendix 4. All subsequent leaf areas were calculated from leaf length using the table as the standard.

(d) the dry weight of green and necrotic leaves.

In the field, white clover plants obtain nitrogen both from soil and by fixation and the level of mycorrhizal

infection may vary with the environmental conditions. Therefore the data presented here is the mean from the mycorrhizal and nitrogen treatments. More detailed information is given in Appendix 5.

RESULTS

The two most obvious components of yield are the number of leaves and the weight of a single leaf + petiole (Table 16). In this experiment 85% of the variation in yield was explained by variation in the number of green leaves, whereas differences in the weight of green leaves explained only 24% of the variation in yield (Table 16).

Differences in the number of green leaves may be caused by different rates of leaf production or by the same rate of leaf production with a different rate of senescence. The total number of leaves produced between harvests 1 and 2 was most affected by the level of phosphorus applied (Table 17), although the 160 kg P/ha plants which received 40 kg K/ha had fewer leaves than plants given 160 kg K/ha. The percentage of green leaves per plant was little affected by the level of phosphorus fertilizer applied but the treatment with the least proportion of green leaves was 160 kg P/ha:40 kg K/ha, which suggests that, where phosphorus is adequate for growth, deficiency of potassium results in a shorter life of leaves (Table 17).

Although the weight of a single leaf was little affected by the fertilizer treatment, the area of the leaves differed. There were significant increases in

Table 16. The total dry matter yield of green leaves (mg/pot), the number of green leaves and the mean weight of a single leaf of white clover grown in Lephinmore peat given different levels of phosphorus and potassium fertilizer (Harvest 2, Experiment 2)

	Treatment					Variation explained when regressed on yield (%)
	PO : KO	P40 : K40	P40 : K160	P160 : K40	P160 : K160	
Total dry matter yield of green leaves (mg/pot)	62	397	306	591	1189	-
Number of green leaves (per pot)	7	32	27	55	101	85
Mean weight of a single leaf (mg)	9	12	11	11	12	24

Table 17. The total number of leaves, and the percentage of green leaves on white clover plants grown in Lephinmore peat when given different levels of phosphorus and potassium fertilizer (Harvest 2, Experiment 2)

	Treatment					SED (76 df)
	PO:KO	P40:K40	P40:K160	P160:K40	P160:K160	
Total no. of leaves (per pot)	10	35	30	83	113	4.5
Percentage of green leaves (per pot)	75	91	90	66	89	4.1

Table 18. The mean weight (mg), length of the central leaflet (mm) and leaf area (cm^2) of a single leaf of white clover given different levels of phosphorus and potassium fertilizer when grown in peat, and the total green leaf area per pot (Harvest 2, Experiment 2)

	Treatment					SED (76 df)
	PO:KO	P40:K40	P160:K160	P160:K40	P160:K160	
Weight of a single leaf (mg)	9	12	11	11	12	0.8
Length of central leaflet (mm)	7	11	11	12	14	0.5
Leaf area of a single leaf (cm^2)	1.2	2.3	2.3	2.8	3.5	0.15
Leaf area of green leaves (cm^2/pot)	8	74	62	154	354	16.1

the length of the central leaflet when adequate amounts of both phosphorus and potassium were given (Table 18). The small increase in leaf length resulted in a larger proportional increase in leaf area. When the total leaf area of green leaves per pot was calculated, the plants which received 160 kg P/ha, 160 kg K/ha had an area of 354 cm^2 which was five times greater than plants which received 40 kg P/ha at either potassium level (74 and 62 cm^2 respectively) and twice the area of plants receiving 160 kg P/ha but 40 kg K/ha (154 cm^2) (Table 18).

Therefore phosphorus, and to a lesser extent potassium, affect the rate at which leaves are produced, and potassium affects the length of life of the leaf. For the maximum area of an individual leaf, adequate supplies of both phosphorus and potassium are required.

The experiment which is described next investigates the response from an established ryegrass/white clover pasture in the field to various nutrients applied as a top dressing.

EXPERIMENT 3. Maintenance fertilizers for an established ryegrass/white clover pasture on peat

EXPERIMENTAL

In 1976, the white clover in a pasture sown in 1972 at the Lephinmore Research Station was chlorotic and grew very slowly. Before sowing, the area had received 5 tonnes lime/ha, 20 kg nitrogen/ha, 60 kg phosphorus/ha as basic slag, 75 kg potassium/ha and trace elements: no

fertilizers were applied in the intervening years. Various trace element deficiencies were suggested as the cause of the poor growth and appearance of the clover plants. Plots, each of 2 x 2 m, were pegged out in the area and several top dressings broadcast on 11 August 1976. The treatments were:-

Lime	@ 2.5 tonnes/ha
Phosphorus	@ 40 kg/ha as superphosphate
Potassium	@ 80 kg/ha as muriate of potash
Molybdenum	@ 0.5 kg/ha as sodium molybdate
Lime + Molybdenum	
Lime + trace elements	(see p.45 for the constituents of the trace element solution)
Lime + phosphorus + potassium	
Lime + phosphorus + potassium + trace elements	
No fertilizer	

The plots were harvested by cutting herbage from a quadrat (area 0.18m²) placed in a random position in each plot. The harvests were taken on 20 October 1976 and 19 June 1977 when the herbage was hand separated into grass, clover and indigenous species, then dried. The greenness of the leaves was determined from samples taken on 14 September 1976, using the method described by Grant (1971).

RESULTS

About a month after the topdressings were applied, the clover in the plots treated with potassium fertilizer were noticeably greener than those that had received the

other treatments (Personal communication from Mr. G.R. Bolton, officer at the research station). Samples of clover from the plots were taken 9 weeks after fertilizer was applied and the greenness of the white clover from each treatment was compared to the greenness of clover which received no fertilizer (Table 19). Only the clover from treatments which included potassium fertilizers was greenner than the control but the variation between replicates was high and the differences were not significant.

At the first harvest, 10 weeks after the treatments were applied, the yield of clover was significantly greater than the unfertilized control only when treatments included potassium fertilizer. By the second harvest, 10 months after the topdressings were applied, clover responded only to treatments where both phosphorus and potassium were applied (Table 19).

Further evidence for the importance of phosphorus and potassium fertilizers for the maximum growth of clover in peat came from a grazing trial set up at Lephinmore Research Station. Floate et al. (1980) recorded responses to potassium fertilizer when applied with phosphorus but not when applied alone in duplicated plots. The total annual dry matter yield in 1977 when 80 kg P/ha and 100 kg K/ha was applied to a ryegrass/white clover sward in spring reached more than 5000 kg/ha (when the amount eaten by the animal was added to that measured between grazing periods). The average yield from a ryegrass/white clover

Table 19. The effect of various topdressings on the greenness and the yield of white clover in a pasture growing on deep peat (Experiment 3)

Topdressing (applied 11/8/76)	Relative greenness on 14/10/76	Dry matter yield (kg/ha) 20/10/76	19/6/77
No fertilizer	1.00	39	52
Lime	0.82	33	135
Phosphorus	0.84	57	87
Potassium	1.25	211	157
Molybdenum	0.94	65	59
Lime + molybdenum	0.87	45	35
Lime + trace elements	0.89	43	89
Lime + phosphorus + potassium	1.32	185	256
Lime + phosphorus + potassium + trace elements	1.47	235	248
SED (16 df)	0.247	52.9	54.4

pasture at the farm was about 2000-2500 kg/ha. The dry matter yield of white clover was seven times greater when given phosphorus and potassium than the yield from the unfertilized treatment and there was a 3 fold response by ryegrass. Following these observations, experiments were set up in the field to determine more exactly (i.e. with greater replication), the phosphorus and potassium fertilizer requirements of ryegrass/white clover pasture when grazed or cut. The work from the cut experiment is described in more detail in this thesis. The results from the field trial, undertaken by Dr. Floate, HFRO, will be referred to where they are relevant.

EXPERIMENT 4. The response to phosphorus and potassium fertilizer by a ryegrass/white clover pasture on deep peat

EXPERIMENTAL

The experiment was set up on the abandoned site of a *Rhizobium*/white clover field trial (Newbould *et al.*, 1980). There were 32 plots, each 4 x 2 m, and in the year before the site was taken over for the phosphorus/potassium fertilizer trial there was no difference in yield attributable to the original treatments. The trial was sown in June 1975 and received 7.5 tonnes lime/ha, 20 kg P/ha as ground mineral phosphate, 20 kg P/ha as superphosphate, 60 kg K/ha as muriate of potash. Half of the plots received 30 kg N/ha as nitram and all received the trace element solution described above. In spring 1976 and 1977, the plots were dressed with 40 kg P/ha as

superphosphate and 40 kg K/ha as muriate of potash. The plots were sown with 20 kg/ha perennial ryegrass cv. Perma and 3 kg/ha white clover cv. Grasslands Huia.

The treatments applied to the phosphorus/potassium fertilizer experiment were restricted by the number of plots (32), by the need for four replicates and because this cut plot experiment was linked to treatments given in the adjoining grazed experiment. The result was an incomplete factorial experiment in which the treatments were:-

(1)	0	kg P/ha	0	kg K/ha	)	
(2)	0	"	50	"	)	as grazed experiment
(3)	30	"	0	"	)	
(4)	30	"	50	"	)	
(5)	30	"	100	"		
(6)	40	"	50	"		
(7)	40	"	100	"		
(8)	50	"	150	"		

After the first seven treatments were allocated, it was decided to apply a greater dressing of fertilizer, 50 kg P/ha and 150 kg K/ha, to the remaining treatment rather than fill one of the missing treatments of the factorial. Phosphorus and potassium were applied as superphosphate and muriate of potash on 3 May 1978. The plots were to be harvested at the end of each of the three grazing periods planned for the companion experiment. However, there was insufficient growth from the cut plots after the second grazing period to provide

another sample of herbage. Samples of pasture were taken from 0.64 m^2 quadrats chosen at random from two of eight positions in the plot and the whole plot was then trimmed to a height of 2 cm. Subsamples of herbage (1/20th fresh weight) were taken for hand separation into clover, ryegrass and indigenous species after thoroughly mixing the sample. The total sample was dried and the percentage of each component in the subsample was applied to the total sample and multiplied up to give yield in kilograms/hectare.

RESULTS

(a) The cut experiment

A photograph of the site, taken in August 1978, is shown in Fig. 12.

The annual dry matter yield in 1978 from the cut plots ranged from 1275 kg/ha where no fertilizer was applied to 3564 kg/ha where the greatest dressing of fertilizer was applied (50 kg P/ha, 150 kg K/ha). The mean botanical composition of dry matter was 64% white clover, 19% perennial ryegrass and 17% indigenous species. The dry matter yield for 1978 of each component of the pasture for each treatment is given in Table 20. The yields at the two sampling times are given in Appendix 6.

Overall there was a 2 fold response by white clover to phosphorus from 0-30 kg P/ha, a 4 fold response to potassium up to 150 kg K/ha, and a synergistic effect on yield with 30 kg P/ha, 50 kg K/ha. By comparison,

Table 20. The annual dry matter yields (kg/ha) of herbage from an established ryegrass/white clover pasture topdressed with various levels of phosphorus and potassium fertilizer when the pasture was cut and removed (Experiment 4)

Level of phosphorus	0	0	30	30	30	40	40	50	SED
potassium	0	50	0	50	100	50	100	150	(21 df)
White clover	491	524	522	1605	2290	1482	2264	2692	174.8
Ryegrass[†]	246	284	524	558	452	502	440	578	88.1
Indigenous species[†]	538	491	419	401	330	361	391	294	126.2
Total	1275	1299	1465	2564	3072	2345	3095	3564	149.6

[†] Data for ryegrass and indigenous species shown for comparison

ryegrass responded 1 fold to 30 kg P/ha but there was no response to potassium. The yield from indigenous species declined as the quantity of fertilizer in the topdressing increased.

The yields of clover with each combination of phosphorus and potassium fertilizer are shown in Fig. 13. In addition, the anticipated yields for the missing treatments from the factorial design of the experiment are drawn. It was assumed that (a) there was no response to phosphorus in the absence of potassium and vice versa: (b) the yield from 50 kg P/ha with 150 kg K/ha was the maximum: (c) there was no response to phosphorus greater than 30 kg P/ha in the missing treatments.

White clover did not respond to a dressing of phosphorus greater than 30 kg P/ha, possibly because 40 kg P/ha had been applied in the previous four years (see Table 4). The response to potassium was tailing off with 150 kg K/ha but there may have been significant response from dressings greater than this level.

(b) The adjoining grazed experiment

The annual dry matter yields (kg/ha) minus the growth which would have taken place over the three grazing periods in June, August and September (of 2, 4 and 2 weeks duration respectively) for the grazed experiment are given in Table 21. The data was collected by Mr. G.R. Bolton, Lephinmore research farm, for Dr. M.J.S. Floate. The composition of the sward was different from the cut experiment. Here the mean botanical composition of

Figure 12. The site at Lephinmore (Experiment 4)

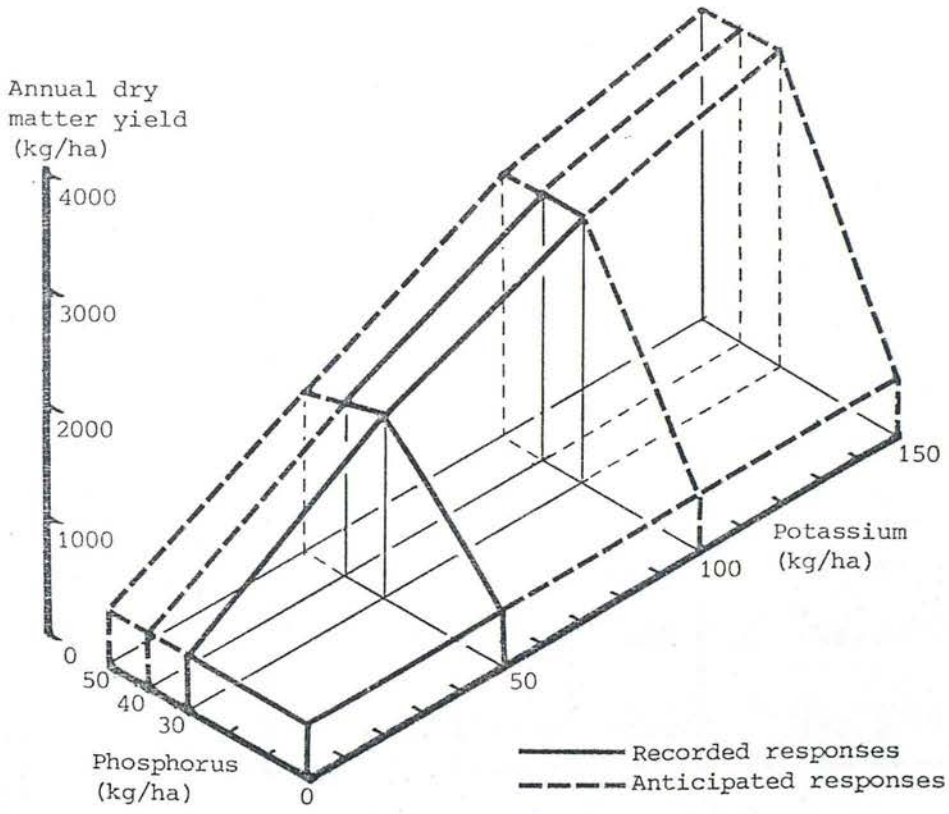


Figure 13. The annual dry matter yields (kg/ha) of white clover from an established ryegrass/white clover pasture on deep peat when combinations of levels of potassium and phosphorus fertilizers were applied in an incomplete factorial design (unbroken lines). The anticipated yields for the missing treatments of the factorial are indicated by broken lines (see text for explanation) (Experiment 4).

the dry matter was 72% ryegrass, 15% white clover and 13% indigenous species and thus there was a reversal in the percentages of clover and ryegrass in the annual yield in the two experiments. There was no response by white clover to phosphorus or potassium fertilizer applied alone, but there was a 1 fold increase in yield over the other three treatments when phosphorus and potassium were applied together but the effect was not significant. Ryegrass responded to phosphorus but not to potassium and there was little effect of treatment on indigenous species.

Table 21. The annual dry matter yields (kg/ha) from an established ryegrass/white clover pasture topdressed with two levels of phosphorus (0 and 30 kg P/ha) and two levels of potassium fertilizer (0 and 50 kg P/ha) in a grazed experiment (by courtesy of Dr. M.J.S. Floate - see Experiment 4)

Level of phosphorus Level of potassium	0 0	0 50	30 0	30 50	SED (5 df)
White clover	463	483	328	703	341.7
Ryegrass	1914	2213	2571	2531	93.6
Indigenous species	451	419	325	400	118.6
Total	2828	3115	3224	3634	374.1

The next section presents the experimental work which measures the effects of added magnesium on two soils, the effects of added calcium carbonate, magnesium and potassium on the deep peat and the interaction between calcium carbonate and the form of phosphorus applied.

THE EFFECT OF CALCIUM CARBONATE, MAGNESIUM AND POTASSIUM ON THE GROWTH AND NUTRIENT CONTENT OF WHITE CLOVER IN PEAT AND THE INTERACTION BETWEEN CALCIUM CARBONATE AND FORM OF PHOSPHORUS

In accordance with the aim of the investigation, to identify and quantify the fertilizer requirements of white clover for the major elements when grown in hill soils, a pot experiment was set up to measure the growth responses in two soils to fertilization with magnesium.

EXPERIMENT 5. The response to magnesium in two soils

EXPERIMENTAL

Magnesium was applied at the rates 0, 10 and 100 kg Mg/ha as magnesium sulphate. To obviate an effect of sulphate as a treatment (magnesium sulphate supplied 0, 13 and 130 kg S/ha) the basal application of potassium was applied as potassium sulphate supplying 300 kg K/ha and 123 kg S/ha, thus satisfying any requirement the clover may have had for sulphur. Sulphate is a suitable variable anion because Mengel (1961) found it was the one anion least likely to affect the uptake of other ions. The two soils used in the experiment, Sourhope brown earth and Lephinmore peat, were limed to pH 5.5 with calcium carbonate. The brown earth required equivalent to 1.37 tonnes lime/ha and the peat required equivalent to 3.47 tonnes lime/ha. Trace elements and 150 kg P/ha, as tricalcium orthophosphate, were applied and the

seedlings were inoculated with rhizobia.

The experiment was arranged in a split plot design with soils as the main plots and magnesium levels as the sub-plots. The plots were sown on 25 September 1975 and harvested 8 and 14 weeks later on 24 November 1975 and 5 January 1976.

RESULTS

The treatments had no effect upon the number of seedlings established 12 days after sowing (Table 22). In both soils, at the first harvest, 100 kg Mg/ha slightly reduced dry matter yield of the shoot but there was no effect of magnesium fertilizer on yield at the second harvest (Table 22).

Table 22. The effect of added magnesium on the number of seedlings established 12 days after sowing and the yield of the shoot (g DM/pot) of white clover grown in two soils and sampled on two occasions (Experiment 5)

	Soil	Magnesium (kg/ha)			SED (5 df-Soils) (16 df-Mg)
		0	10	100	
Seedlings established 12 days after sowing (No./pot)	Brown Earth	37	37	38	0.9
	Peat	37	38	38	
Dry matter yield of shoot (g/pot)					
8 weeks	Brown Earth	3.1	2.9	2.8	0.11
	Peat	1.8	1.5	1.5	
14 weeks	Brown Earth	1.5	1.5	1.8	0.15
	Peat	1.5	1.7	1.6	

Following this experiment an attempt was made to investigate, by growth analysis, the possible synergistic effect on growth of applications of phosphorus and potassium fertilizers in peat where no magnesium fertilizer was applied. Early in the growth analysis experiment the leaves of plants which received the higher level of potassium fertilizer (160 kg K/ha) became red around the margins (Fig. 14) and McNaught and Dorofaeff (1960) described similar colours in leaves of white clover plants deficient in magnesium. The authors suggested 0.12% Mg was the critical concentration in the shoot. Chemical analysis of the leaves confirmed that the magnesium concentration in plants which received 160 kg K/ha was low (0.10%) but the concentration was higher (0.18%) in plants which received 40 kg K/ha. The experiment was abandoned.

In order to identify the conditions where there may be antagonism between cations for uptake by the plant or in nodulation, the following pot experiment was set up in the peat soil with varying levels of magnesium, potassium and lime (as calcium carbonate) in factorial combinations.

EXPERIMENT 6. Calcium carbonate x magnesium x potassium factorial experiment in peat

EXPERIMENTAL

There were four levels of calcium carbonate and potassium and three levels of magnesium fertilizer applied to the Lephinmore peat. Lime, as calcium carbonate, was

Figure 14. Probable symptoms of magnesium deficiency in the leaves of white clover

applied at 0, 1.45, 2.90 and 5.80 tonnes/ha, magnesium sulphate at 0, 36 and 143 kg Mg/ha and potassium chloride at 0, 76, 152 and 304 kg K/ha. In addition, all pots received a basal dressing of phosphorus equivalent to 145 kg P/ha as tricalcium orthophosphate and the trace element solution. There were 48 treatments in the full factorial experiment. The seedlings were inoculated with rhizobia.

The experiment was sown on 21 November 1977 and harvested on 3 occasions, 12, 20 and 29 weeks after sowing on 14 February 1978, 11 April 1978 and 11 June 1978. The data for each treatment at each harvest for seedling establishment, yield of shoot, chemical content of the shoot, are given in Appendices 7, 8 and 9 and the yield of the root, the soil pH, and the number of nodules at harvest 3 are given in Appendix 10.

RESULTS

The establishment of seedlings (Table 23)

Fourteen days after sowing, 32 or more of the 40 seeds sown had germinated and were established. There were two or three fewer plants in pots which did not receive calcium carbonate or potassium fertilizer.

The main effects of the treatments on the yield of the shoot (Table 24)

(a) Calcium carbonate. The response to calcium carbonate varied between harvests. The level giving the greatest yield at harvest 1 was 1450 kg $CaCO_3$/ha: at harvest 2, the

Table 23. The effect of added calcium carbonate, potassium and magnesium on the number of seedlings of white clover established in Lephinmore peat 14 days after sowing (Experiment 6)

Treatment	Level (Kg/ha)	Seedlings established	SED (94 df)
Calcium carbonate	0	33	
	1450	35	0.5
	2900	35	
	5800	35	
Magnesium	0	34	
	36	35	0.4
	143	35	
Potassium	0	32	
	76	35	0.5
	152	35	
	304	34	

Table 24. The effect of added calcium carbonate, magnesium and potassium on the yield (g DM/pot) of shoots of white clover grown in Lephinmore peat and sampled on three occasions (Experiment 6)

Treatment	Level (kg/ha)	Dry matter yield of shoot (g/pot) H1	H2	H3	H1 + 2 + 3
Calcium carbonate	0	1.6	0.7	0.2	2.5
	1450	2.3	3.2	1.9	7.4
	2900	1.7	3.8	3.2	8.7
	5800	0.7	2.8	3.5	7.0
	SED (94 df)	0.05	0.09	0.10	0.12
Magnesium	0	1.5	2.4	1.7	5.6
	36	1.6	2.6	2.0	6.2
	143	1.6	2.5	2.2	6.4
	SED (94 df)	0.04	0.07	0.09	0.10
Potassium	0	0.9	0.6	0.4	1.9
	76	1.7	2.4	1.9	6.0
	152	1.9	3.2	2.6	7.7
	304	1.8	4.1	4.0	10.0
	SED (94 df)	0.05	0.09	0.10	0.12

greatest yield was with 2900 kg $CaCO_3$/ha: and at harvest 3, 5800 kg $CaCO_3$/ha gave the greatest yield. When no calcium carbonate was applied, growth declined with each harvest (from 1.6 to 0.2 g/pot) but, where 5800 kg $CaCO_3$ was applied, yield increased from the least at harvest 1 (0.7 g/pot) to the greatest at harvest 3 (3.5 g/pot). Over the three harvests there was least growth (2.5 g/pot) from plants without calcium carbonate, most growth from plants given 2900 kg $CaCO_3$/ha (8.5 g/pot) and growth was similar when plants were given 1450 and 5800 kg $CaCO_3$/ha.

(b) Magnesium. Magnesium had no effect on growth at harvests 1 and 2 but, at harvest 3, and over the three harvests, there was a small response to 36 kg Mg/ha.

(c) Potassium. Application of potassium increased growth at each harvest. At harvest 1 potassium had no effect on growth above 76 kg K/ha but in the subsequent harvests application of potassium fertilizer up to 304 kg K/ha increased yield.

The main effects of treatment on the yield of roots (Table 25)

The weight of the root was measured at the end of the experiment (harvest 3) and was most like the additive yield from the three harvests.

(a) Calcium carbonate. The weight of the root increased with the level of calcium whereas the additive yield of the shoot from the three harvests was depressed with the greatest level of calcium carbonate (5800 kg $CaCO_3$/ha).

Table 25. The effect of added calcium carbonate, magnesium and potassium on the yield (g DM/pot) of the roots of white clover grown in Lephinmore peat at the third sampling time (Experiment 6)

Treatment	Level (kg/ha)	Dry weight of root at harvest 3 (g/pot)	SED (94 df)
Calcium carbonate	0	1.0	
	1450	1.8	0.10
	2900	2.4	
	5800	3.0	
Magnesium	0	1.9	
	36	2.1	0.09
	143	1.9	
Potassium	0	0.5	
	76	1.9	0.10
	152	2.4	
	304	3.4	

Table 26. The levels of added calcium carbonate and potassium at which white clover grown in peat responded to magnesium fertilizer (Experiment 6 Harvest 3)

Calcium carbonate (kg/ha)	Magnesium (kg/ha)	Potassium (kg/ha) 152 Yield	152 %Mg	304 Yield	304 %Mg	SED (94 df) Yield	SED (94 df) %Mg
2900	0	2.7	0.11	5.2	0.07		
	36	2.9	0.29	6.3	0.15		
	143	3.5	0.64	6.7	0.51		
						0.41	0.11
5800	0	3.1	0.13	3.0	0.12		
	36	3.6	0.29	5.2	0.21		
	143	4.6	0.60	6.7	0.40		

(b) Magnesium. There was a small response by both root and shoot to 36 kg Mg/ha but the yield from the root was decreased with 143 kg Mg/ha.

(c) Potassium. Both root and shoot responded greatly to application of potassium.

Interactions between nutrients on growth

(a) Calcium carbonate x potassium (Fig. 15). The response to potassium was restricted when growth was inhibited either by too little or too much calcium carbonate.

(b) Calcium carbonate x potassium x magnesium (Table 26). There was no response to magnesium at harvest 1 and the response was only apparent at harvest 3 in treatments with the greater levels of calcium carbonate and potassium. The magnesium did not substitute for low levels of potassium or calcium in growth nor was there antagonism between the cations which affected growth.

The concentration of nutrients in the shoots

(a) The effect of calcium carbonate (Fig. 16). The concentration of calcium in the shoots increased with the level of calcium applied except where the higher levels of calcium carbonate were applied at harvest 1 and where the highest level of calcium carbonate was applied in harvest 2. The concentrations of magnesium and phosphorus were depressed with increasing levels of calcium carbonate but the concentration of potassium in the shoots was inversely related to dry matter yield. Concentrations of potassium

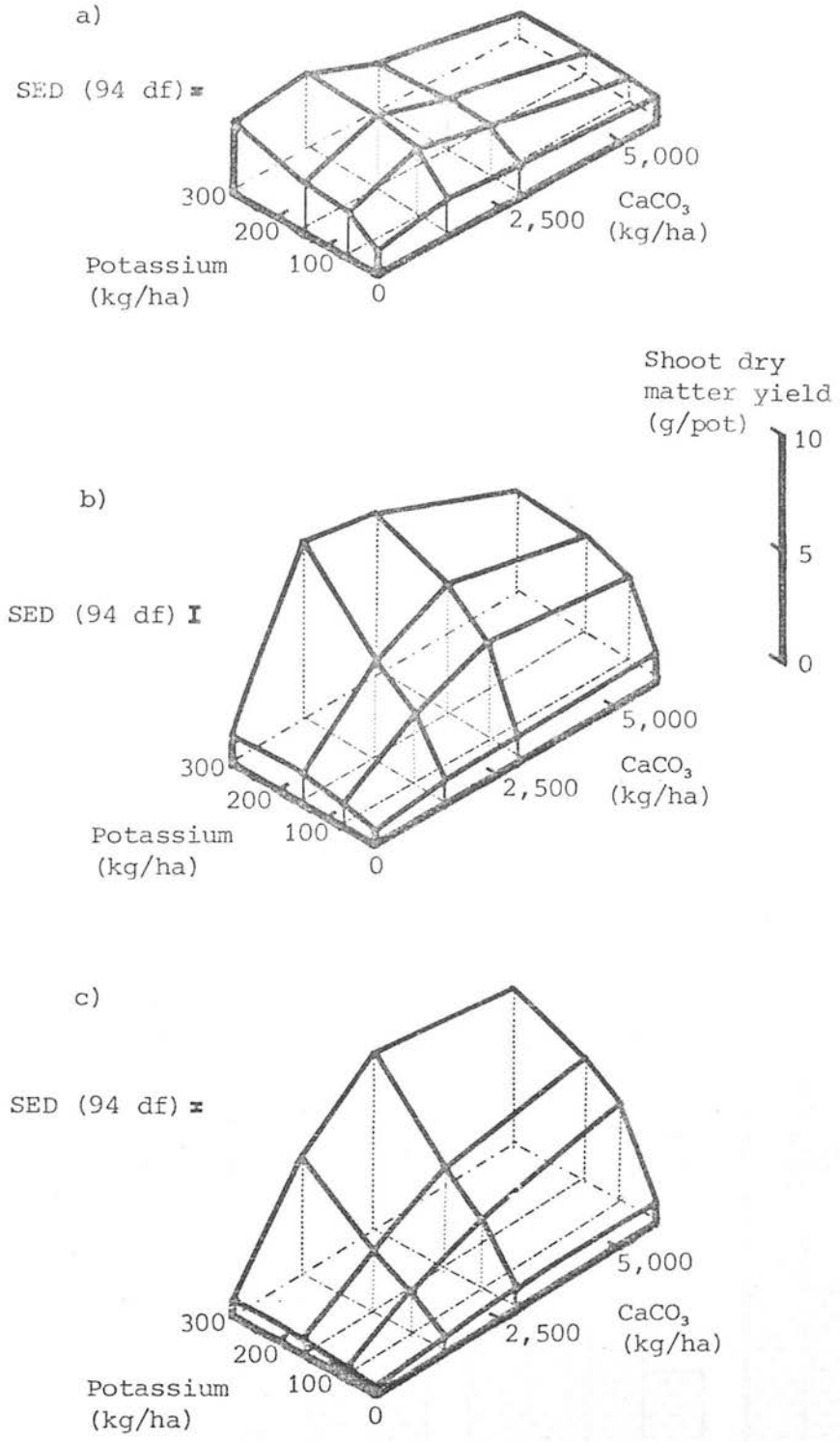


Figure 15. The interaction between added calcium carbonate and potassium on the growth of white clover (shoot dry matter, g/pot) in the Lephinmore deep peat (a) Harvest 1, (b) Harvest 2, (c) Harvest 3.

(Experiment 6)

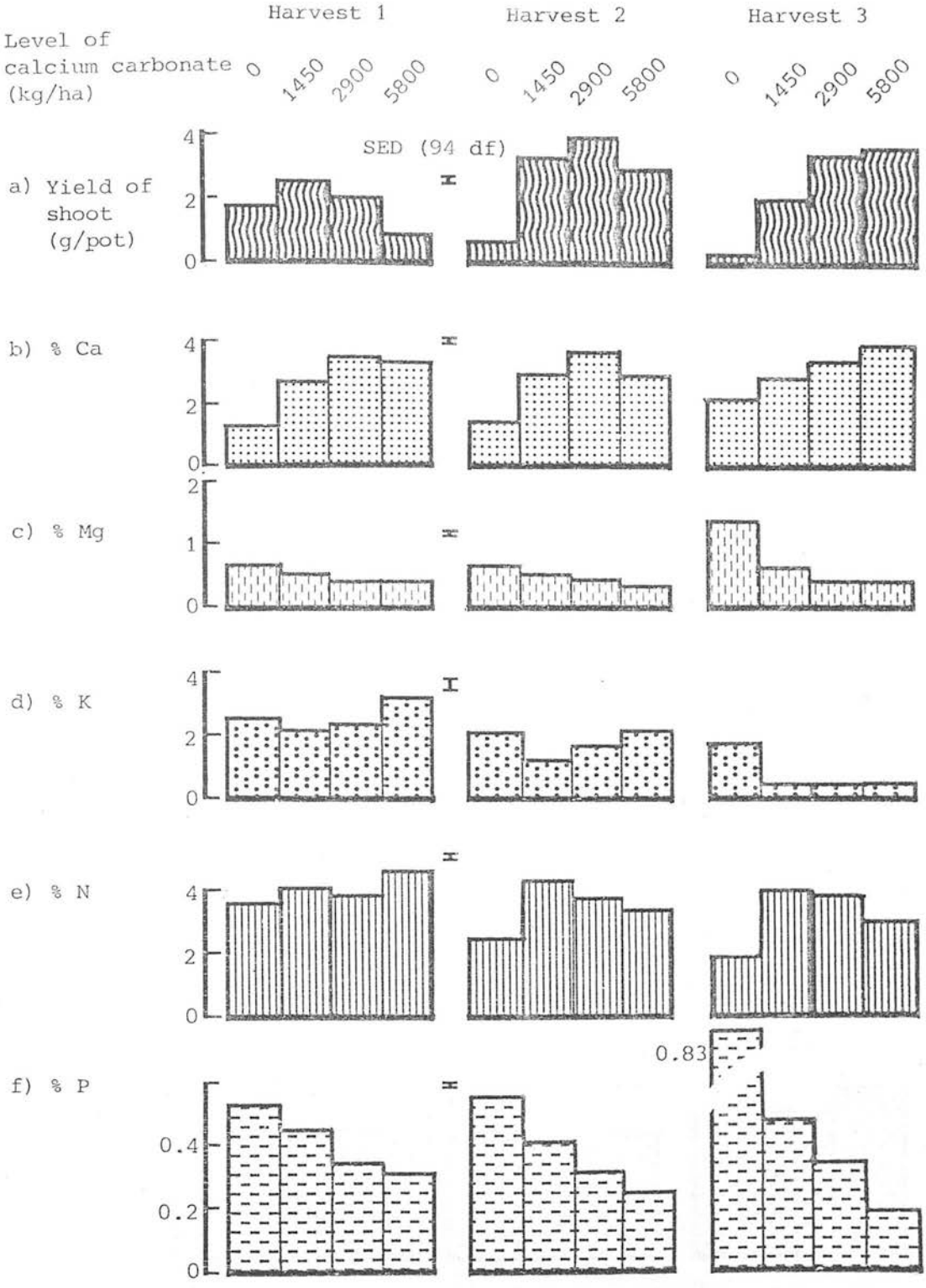


Figure 16. The effects of added calcium carbonate on the yield (shoot dry matter, g/pot) and the concentrations of calcium, magnesium, potassium, nitrogen and phosphorus in the shoots of white clover grown in the Lephinmore deep peat and sampled on three occasions (Experiment 6).

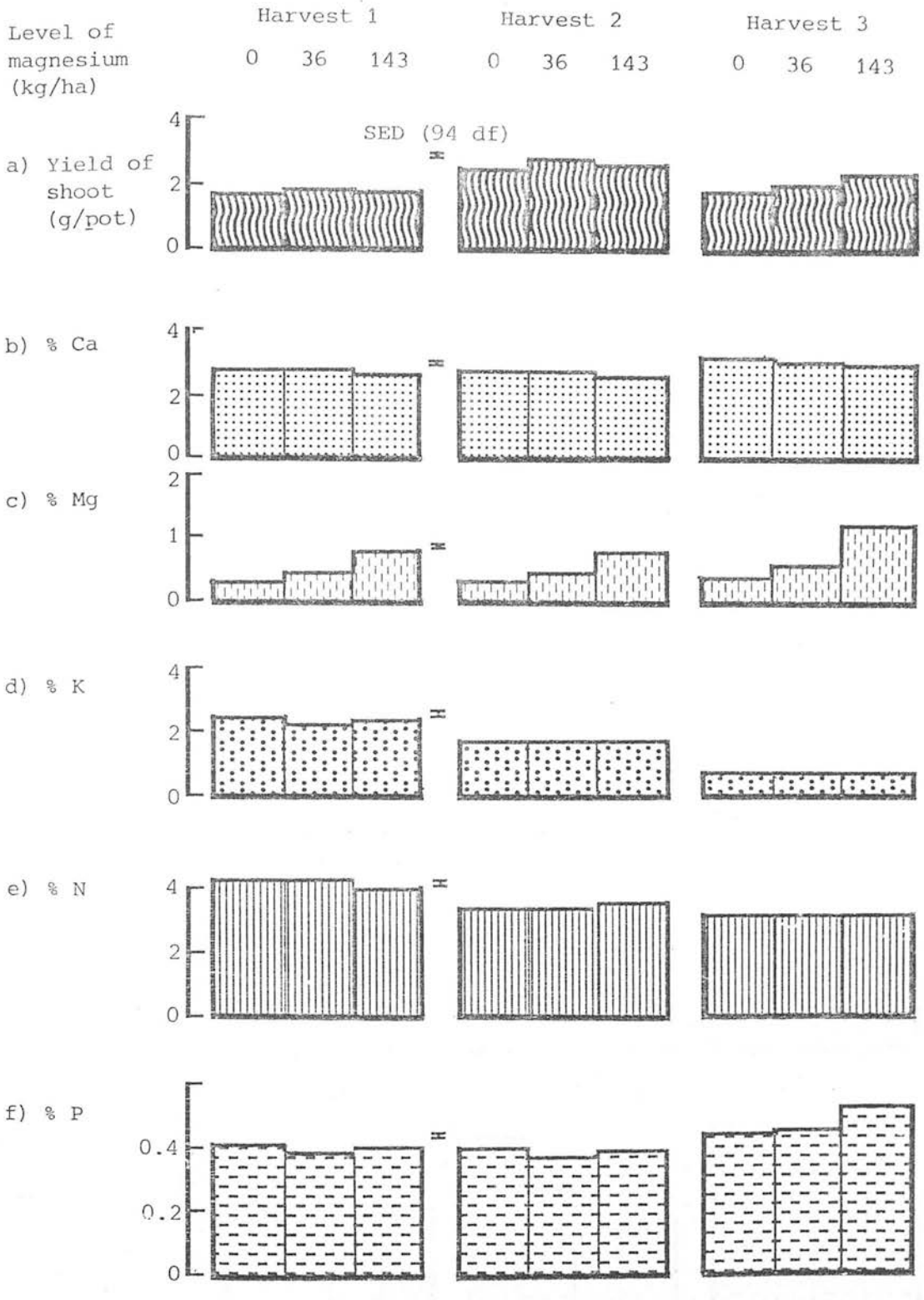


Figure 17. The effect of added magnesium on the yield (shoot dry matter, g/pot) and the concentrations of calcium, magnesium, potassium, nitrogen and phosphorus in the shoots of white clover grown in the Lephinmore deep peat and sampled on three occasions (Experiment 6).

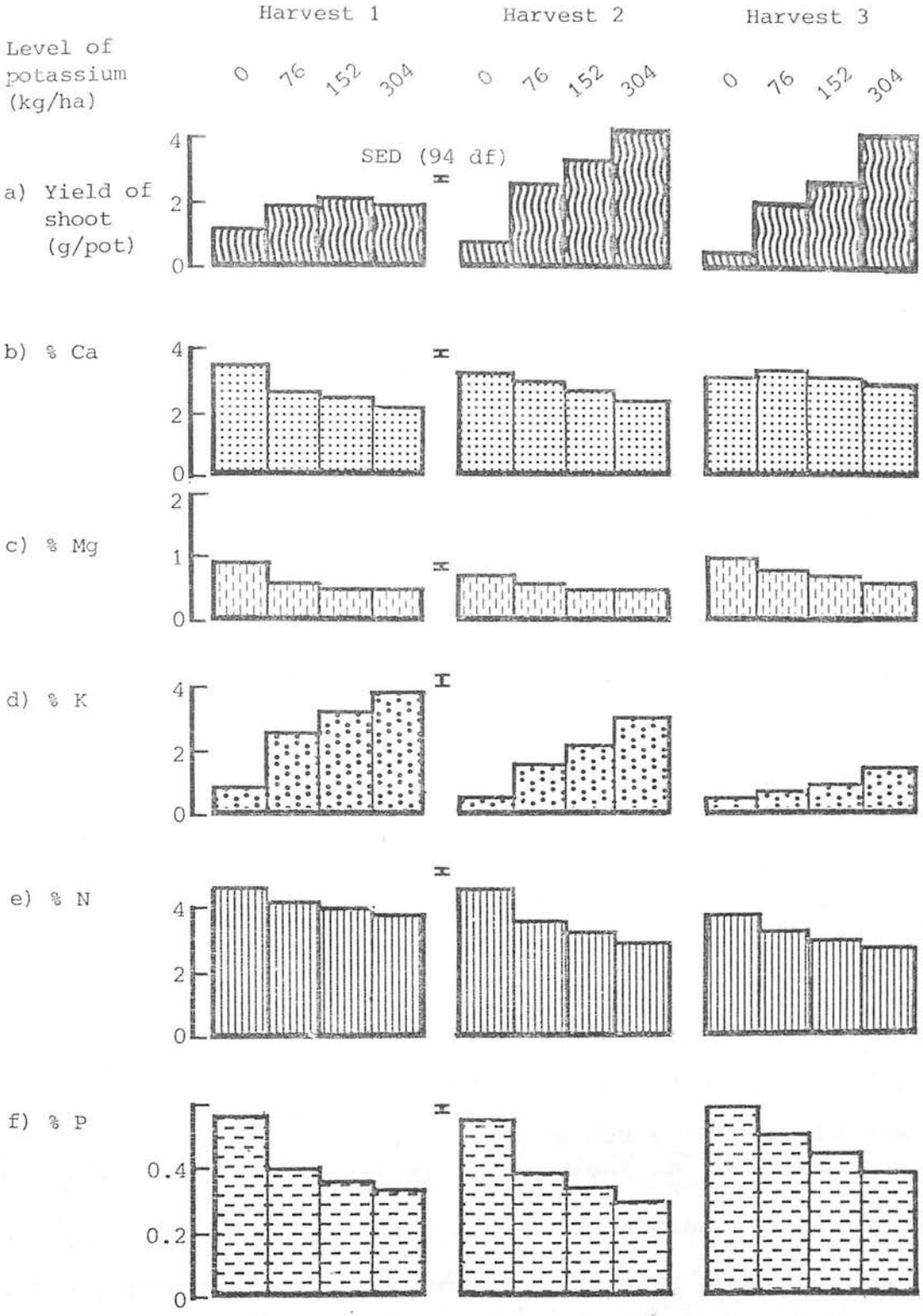


Figure 18. The effect of added potassium on the yield (shoot dry matter, g/pot) and the concentrations of calcium, magnesium, potassium, nitrogen and phosphorus in the shoots of white clover grown in the Lephinmore deep peat and sampled on three occasions (Experiment 6).

were greater where yields were lower. The concentration of nitrogen varied: where no calcium carbonate was applied, the concentration of nitrogen declined over the three harvests and this was probably due to deficiency of nitrogen in the non-nodulated plants (see later section) after the soil and seed nitrogen had run out: in other treatments the concentration of nitrogen increased when growth of the shoots was restricted by treatment with calcium carbonate.

(b) The effect of magnesium (Fig. 17). Application of magnesium had little effect upon the concentrations of any elements in the shoots except magnesium itself.

(c) The effect of potassium (Fig. 18). Application of potassium increased the concentration of potassium in the shoots but the concentrations declined at each harvest. With the decline in the concentration of potassium, there was some increase in the concentration of calcium and magnesium. Increasing levels of added potassium at each harvest decreased the concentrations of calcium, magnesium, nitrogen and phosphorus.

Concentrations of calcium, magnesium and potassium in the shoots below which plants were deficient in the element

(a) Calcium. The identification of calcium deficiency in the experiment is confounded by the poor nodulation in plants grown in peat without calcium carbonate (see later section) and by the depression in growth caused by the higher levels of calcium carbonate. Where nitrogen was sufficient, and where the growth response to calcium

increased, the information in Fig. 16 suggests the deficiency may occur at about 1% calcium in the shoot.

(b) Magnesium. The greatest responses to magnesium occurred at harvest 3 with 2900-5800 kg $CaCO_3$/ha and 152-304 kg K/ha (Table 26). The concentration of magnesium in the shoots above which there was no increase in growth was greater than 0.29%.

(c) Potassium. Measurements from harvests 1 and 3 (Fig. 18) support the results in Experiment 1 that responses to potassium occur when the concentration in the shoots is below 1%, but the data from harvest 2 suggest clover will still respond to potassium when the concentration in the shoot is between 1-2%.

Nodulation (Table 27)

The number of nodules on the roots of clover were counted at the end of the experiment.

(a) Calcium carbonate. The most striking result was the lack of nodulation in the absence of calcium carbonate. There were fewer nodules on plants with 1450 kg $CaCO_3$/ha than with the higher levels.

(b) Magnesium. The absence of magnesium slightly reduced the number of nodules formed on the root.

(c) Potassium. There was an increase in the number of nodules on the roots of plants given potassium fertilizer. However, increasing levels of potassium decreased the number of nodules per unit weight of root.

(d) Interactions. There were no significant interactions.

Table 27. The effect of added calcium carbonate, magnesium and potassium on the number of nodules formed on roots of white clover grown in Lephinmore peat at the third sampling time (Experiment 6)

Treatment	Level (kg/ha)	Number of nodules per pot	Number of nodules per g DM root
Calcium carbonate	0	0	0
	1450	100	63
	2900	182	95
	5800	222	96
	SED (94 df)	15.0	7.1
Magnesium	0	99	55
	36	122	67
	143	140	74
	SED (94 df)	13.9	7.0
Potassium	0	52	91
	76	134	66
	152	151	56
	304	166	41
	SED (94 df)	15.0	7.1

Table 28. The effect of added calcium carbonate, magnesium and potassium on the pH of the Lephinmore peat at sowing and at the end of the experiment (Experiment 6)

Treatment	Level (kg/ha)	pH of soil sowing	pH of soil final harvest
Calcium carbonate	0	3.9	3.5
	1450	4.9	4.0
	2900	5.5	4.5
	5800	7.0	5.5
	SED (94 df)	0.01	0.02
Magnesium	0	-	4.5
	36	-	4.4
	143	-	4.3
	SED (94 df)	-	0.01
Potassium	0	-	4.6
	76	-	4.4
	152	-	4.3
	304	-	4.2
	SED (94 df)	-	0.02

The pH of the soil (Table 28)

At the beginning of the experiment with the calcium carbonate treatments, the pH of the soil ranged from 3.9-7.0 but, at the end of the experiment, the range was narrower, pH 3.5-5.5. The pH of the soil had dropped more on treatments with the higher levels of lime (Table 28). At the end of the experiment, there was a pH drop of 0.2 and 0.4 units respectively with the magnesium and potassium treatments.

It is suggested that the depression in yield when high levels of calcium carbonate were applied was caused by induced phosphorus deficiency, because the dissolution of the applied tricalcium orthophosphate was inhibited in the limed soil. The following experiment compares the growth and nodulation of white clover in the Lephinmore peat, given the same levels of calcium carbonate as Experiment 6 with 145 kg P/ha as tricalcium orthophosphate or as the more soluble monocalcium orthophosphate.

EXPERIMENT 7. The interaction between the form of phosphorus fertilizer and rate of applied calcium carbonate

EXPERIMENTAL

The rates of lime were 0, 1.45, 2.90 and 5.80 tonnes/ha as calcium carbonate. 145 kg P/ha was given as tricalcium orthophosphate ($Ca_3(PO_4)_2$ (solubility 2×10^{-3} g/100 ml cold water) or as monocalcium orthophosphate $Ca(H_2PO_4)_2 . H_2O$ (solubility 1.8 g/100 ml cold water). Trace elements and 304 kg K/ha as potassium chloride were applied as

basal dressings and the seedlings were inoculated with rhizobia.

The experiment was sown on 3 August 1979 and harvested on 14 September 1979, 6 weeks after it was sown.

The concentration of calcium and phosphorus in the soil solution during germination (1 week after fertilizing) was estimated in soil given no lime and 5800 kg lime/ha with the two forms of phosphorus. To 100 g of partially dried peat (fertilized 7 days previously) was added 100 ml of water and the mixture was then shaken for an hour. 100 g of the sludge was centrifuged at 3500 rpm for 30 minutes then filtered through No. 42 Whatman Filter Paper. The solution was analysed for calcium by atomic absorption/emission spectrophotometry and phosphorus by formation of a blue phospho molybdate complex with $SnCl_2$ as the reductant. The relative concentrations of calcium and phosphorus in the supernatant were taken to be similar to the relative concentrations of the two elements in soil solution.

The numerical values for the information presented in the graphs are given in Appendix 11.

RESULTS

Establishment of seedlings (Fig. 19, Table 29)

Thirteen days after sowing, the number of seedlings established (35-39) was unaffected by either form of calcium phosphate when 1450 or more calcium carbonate was applied. In the absence of calcium carbonate very few seeds germinated and established (8 out of 40) when $Ca(H_2PO_4)_2.H_2O$ was applied, but more seedlings

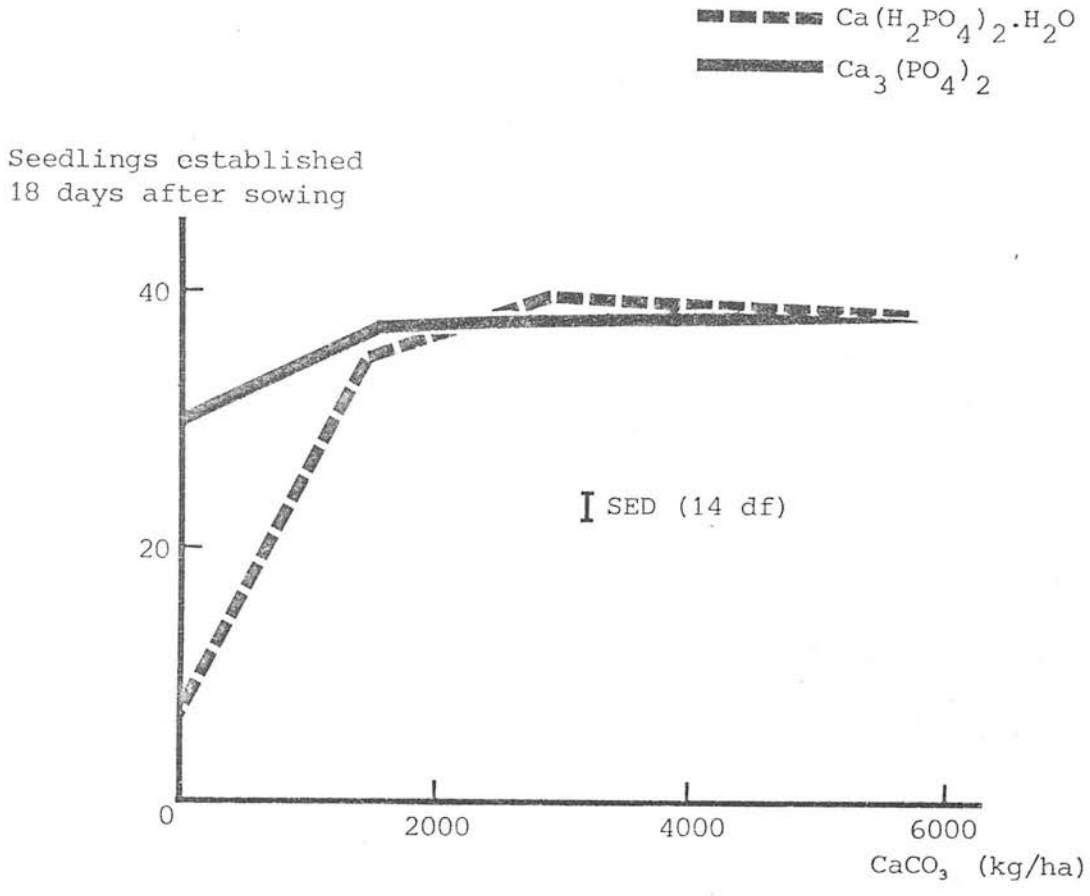


Figure 19. The effect of added calcium carbonate and two forms of phosphorus on the number of seedlings of white clover established in the Lephinmore deep peat thirteen days after sowing (Experiment 7).

Table 29. The effect of two forms of applied phosphorus on the germination of seedlings of white clover in unlimed peat (Experiment 7)

Lime	Phosphorous	Percentage seeds germinated (10 days)
None	None	43
None	$Ca(H_2PO_4)_2$	55
None	$Ca_3(PO_4)_2$	90

Table 30. The concentration of nutrients in seed of white clover cv Grasslands Huia (Experiment 7)

	% N	% P	% K	% Ca	% Mg
Grasslands Huia seed	5.10	0.63	1.37	0.14	0.24
Level of nutrient in the shoot of the mature plant when growth is not limited by the element	>2.3[1]	0.20[2]	1.0[3]	1.0[4]	0.30[5]

1 Experiment 1 of this thesis
2 Experiment 1 of this thesis
3 Experiment 1 of this thesis
4 Experiment 6 of this thesis
5 Experiment 6 of this thesis

established (30 out of 40) when $Ca_3(PO_4)_2$ was given. To decide whether $Ca(H_2PO_4)_2.H_2O$ inhibited establishment or whether $Ca_3(PO_4)_2$ enhanced establishment, seeds were germinated in unlimed peat (given KCl as in the experiment) in an incubator for 10 days when given no phosphate, and the two forms of calcium phosphate. $Ca_3(PO_4)_2$ enhanced germination because 90% of the seeds germinated but only 43% of the seeds germinated in the absence of phosphate and 55% of the seeds germinated in the presence of $Ca(H_2PO_4)_2.H_2O$.

Nutrients in white clover seed (Table 30)

Chemical analysis of clover seed revealed that, of the elements measured, the one in the least concentration in the seed was calcium. The requirement by the mature plant was very much greater than the concentration in the seed. In the unlimed peat, the only source of calcium for growth was from calcium phosphate; $Ca_3(PO_4)_2$ would supply three times more calcium than $Ca(H_2PO_4)_2.H_2O$.

The concentration of calcium in soil solution (Table 31)

The relative concentration of calcium in solution in the unlimed soil during germination (1 week after mixing) was estimated. The concentration of calcium in the solution given $Ca_3(PO_4)_2$ was greater than in the solution given $Ca(H_2PO_4)_2.H_2O$.

Yield of the shoots (Fig. 20)

The yields from the unlimed treatments were less than from plants which received 1450 kg calcium carbonate/ha and can be explained by the fewer number of seedlings

Table 31. The effect of two forms of calcium phosphate and two levels of calcium carbonate on the concentration of calcium and phosphorus in soil solution (ppm) (Experiment 7)

	Phosphorus treatment	Calcium carbonate (kg/ha) 0	5800	SED (10 df)
Calcium	None	96	274	15.2
	$Ca(H_2PO_4)_2H_2O$	114	338	
	$Ca_3(PO_4)_2$	167	311	
Phosphorus	None	0.1	-†	0.05
	$Ca(H_2PO_4)_2H_2O$	2.0	-	
	$Ca_3(PO_4)_2$	1.1	-	

† The solution extracted from the limed peat contained brown colloidal substances which made it impossible to measure the concentration of phosphorus

Table 32. The effect of two forms of calcium phosphate and four levels of calcium carbonate on the concentration of phosphorus in the shoots (Experiment 7)

	Phosphorus in shoots (%) Calcium carbonate (kg/ha) 0	1450	2900	5800	SED (14 df)
$Ca(H_2PO_4)_2H_2O$	-	0.42	0.35	0.23	0.025
$Ca_3(PO_4)_2$	0.39	0.35	0.20	0.17	

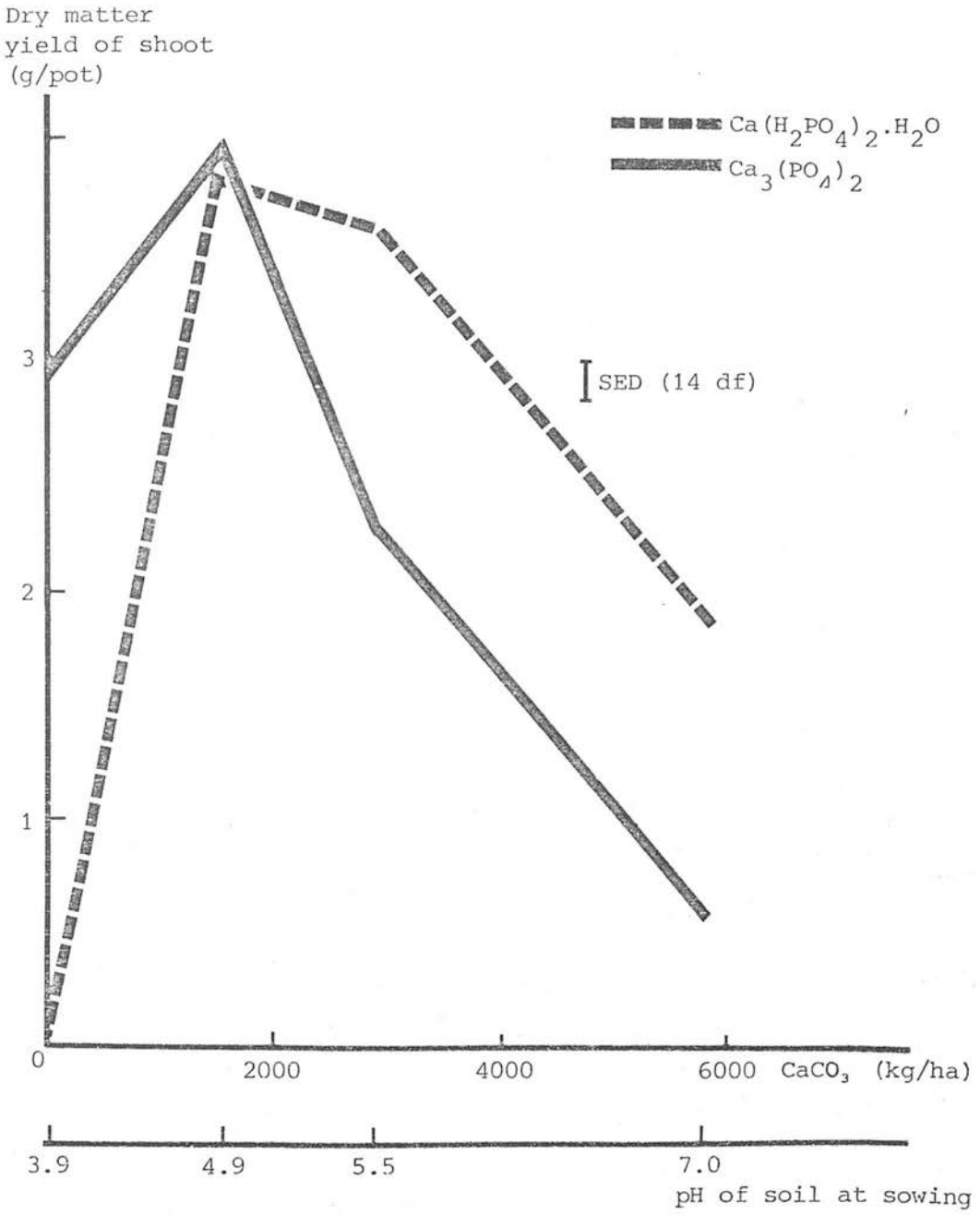


Figure 20. The effect of added calcium carbonate and two forms of phosphorus on the yield of the shoots of white clover grown in the Lephinmore deep peat (Experiment 7).

Ia Ib Ic Id

IIa IIb IIc IId

Figure 21. White clover plants, given four levels of calcium carbonate and two forms of phosphorus, just before they were harvested (Experiment 7)

I $Ca_3(PO_4)_2$

II $Ca(H_2PO_4)_2H_2O$

a 0 $CaCO_3$

b 145 kg $CaCO_3$/ha

c 2700 kg $CaCO_3$/ha

d 5800 kg $CaCO_3$/ha

which established. The growth rate of plants which received levels of calcium carbonate greater than 1450 kg/ha must have been less than those which received 1450 kg $CaCO_3$/ha for there to be a depression in yield. The depression in growth was not as great when $Ca(H_2PO_4)_2.H_2O$ was the form of phosphate applied. A photograph of the plants just before harvest is shown in Fig. 21.

The concentration of phosphorus in the shoots (Table 32)

The concentration of phosphorus in the shoot was less when $Ca_3(PO_4)_2$ was the source of phosphorus at all levels of calcium carbonate, but the differences would only be expected to show up in growth when the concentrations of phosphorus were less than the critical concentration. If the critical concentration is 0.2% (Experiment 1), then from the data, growth reductions would be expected with 5800 kg $CaCO_3$/ha and $Ca_3(PO_4)_2$ and possibly with 2900 kg $CaCO_3$/ha and $Ca_3(PO_4)_2$.

Yield of root (Fig. 22).

The treatments affected root growth similarly to shoot growth but the relative differences in yield between treatments were not so great as with the shoots.

Morphology of the root (Fig. 23)

With $Ca(H_2PO_4)_2.H_2O$, in the absence of lime there was very little root growth, but with $Ca_3(PO_4)_2$ the roots grew fairly well. However, roots in the unlimed soil were browner and the individual roots were thicker and more profusely branched when compared with roots grown in peat

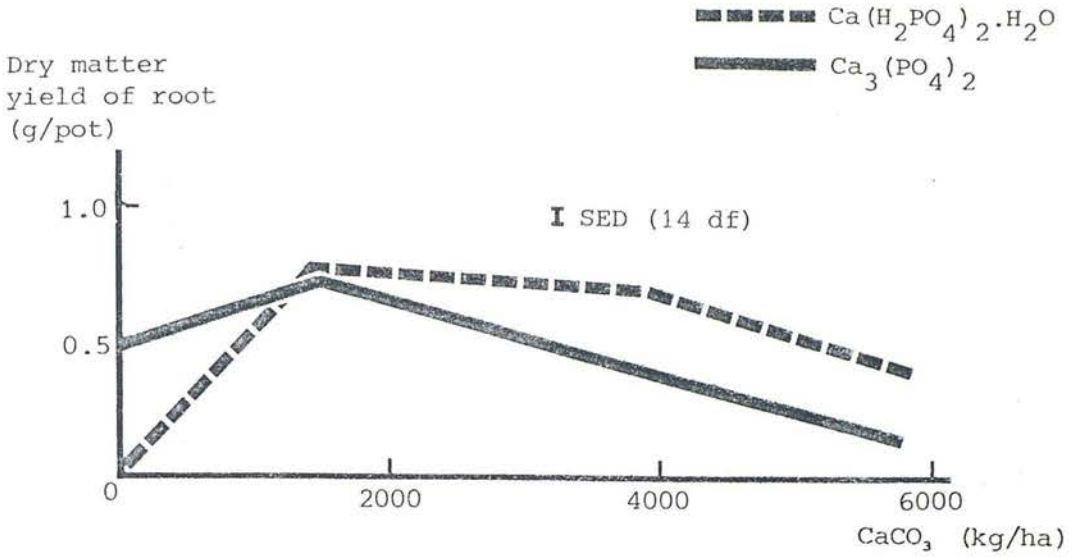


Figure 22. The effect of added calcium carbonate and two forms of phosphorus on the yield of the roots of white clover grown in the Lephinmore deep peat (Experiment 7).

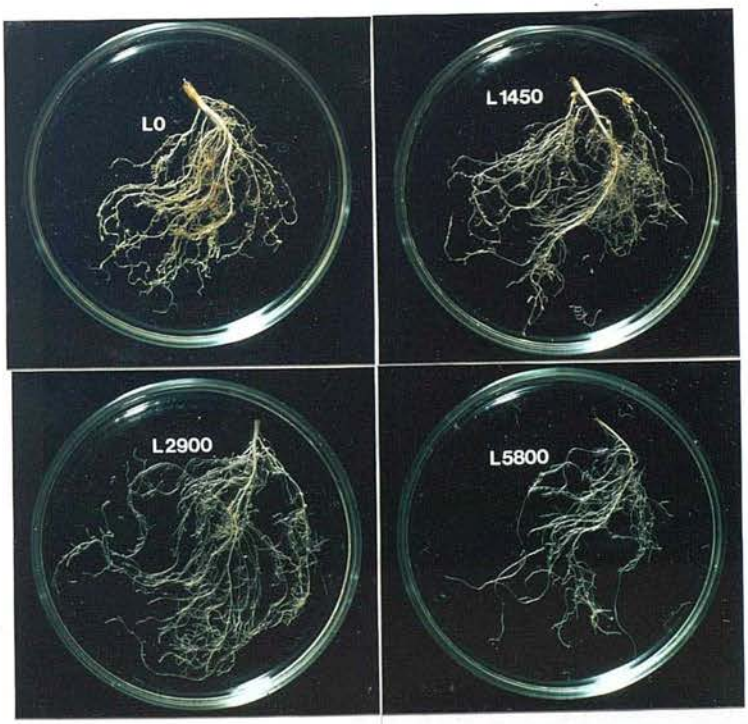


Figure 23. Roots of white clover given four levels of lime (calcium carbonate) (Experiment 7)

given lime at all three rates.

The number and size of root nodules (Fig. 24)

There were no nodules where no lime was applied. There were fewer nodules on plants given 1450 kg $CaCO_3$/ha than plants given 2900 kg $CaCO_3$/ha. The fewer nodules on the root system of plants grown at 5800 kg $CaCO_3$/ha compared with plants given 2900 kg $CaCO_3$/ha was caused by the restricted root growth rather than fewer nodules per unit weight of root. Most nodules occurred on plants given $Ca(H_2PO_4)_2.H_2O$ at 2900 kg $CaCO_3$/ha but they were mainly very small in size (< 1 mm). The treatments which gave the greatest number of nodules longer than 1 mm were with either form of phosphate at 1450 kg $CaCO_3$/ha.

The concentration of phosphorus in the soil solution (Table 31)

Differences in the concentration of phosphorus in solution with 5800 kg $CaCO_3$/ha were not detectable in soil mixed with fertilizers for 1 week (Table 28) but, in the absence of calcium carbonate, the concentration of phosphorus in solution was greatest with $Ca(H_2PO_4)_2.H_2O$ (2 ppm), about half as concentrated with $Ca_3(PO_4)_2$ (1.1 ppm), and was less than 0.1 ppm when no phosphate was applied.

The results from the nutrient experiments from both experimental sections in this part of the thesis will be discussed in the next section.

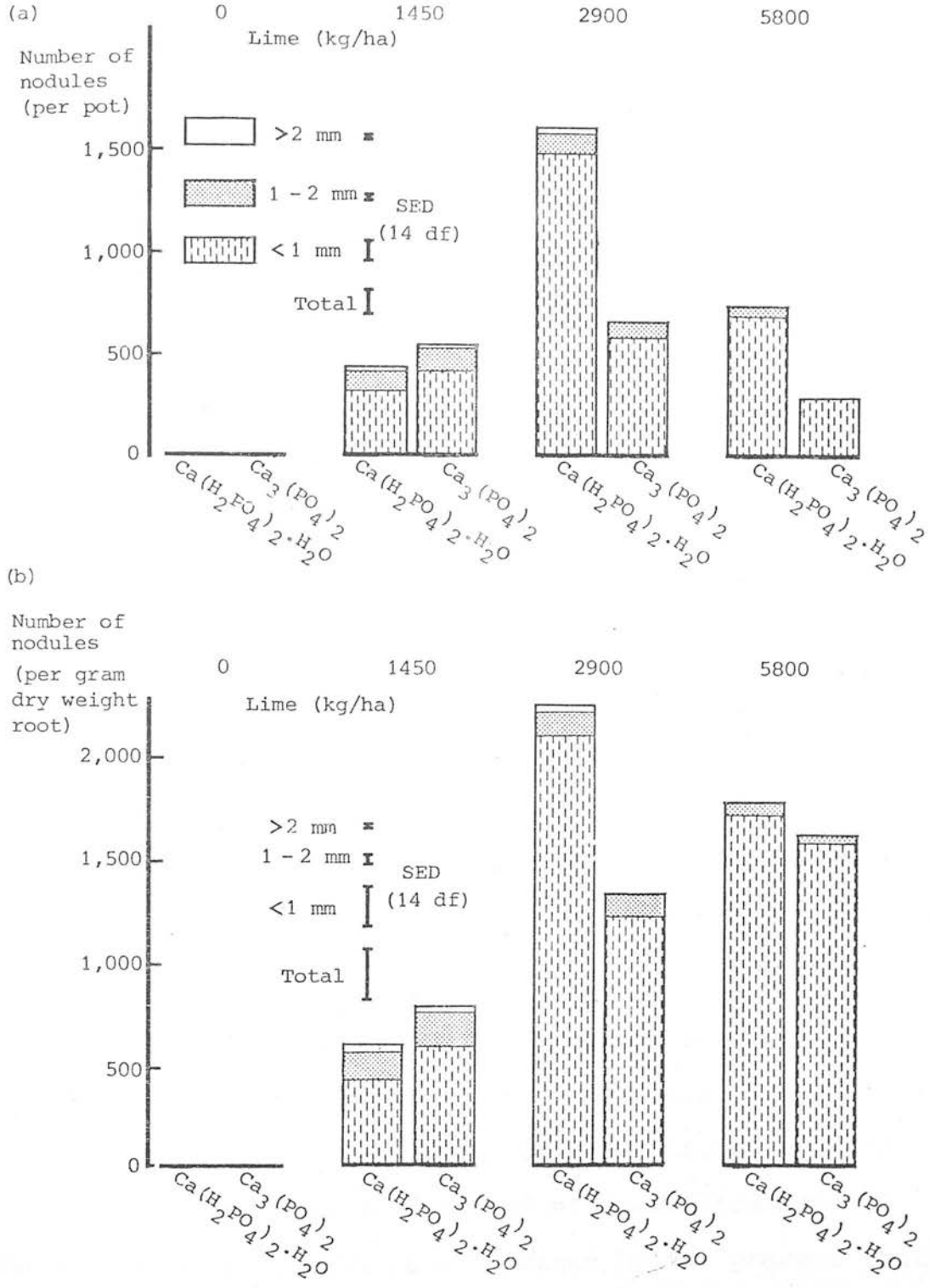


Figure 24. The effect of four levels of calcium carbonate and two forms of phosphorus on the number and size of nodules formed on the roots of white clover (a) nodules/pot (b) nodules/g root (Experiment 7).

GENERAL DISCUSSION

The glasshouse experiments have identified the magnitude of the responses by nodulated white clover, during establishment, to fertilization with the major nutrient elements in three hill soils and the concentrations of nutrients in the shoot below which plant growth was restricted. The detailed examination of clover growth in a deep peat and a brown earth in Experiment 1 has shown that the responses were broadly similar, although their magnitude depended upon the time of the year that the plants were grown and the level of available nutrients in the soil. It seems probable that the relationships between nutrients and the principles established in this study can be applied to the range of hill soils. The work, after Experiment 1, was directed particularly at the deep peat because this soil type was the most acid with the lowest available nutrient content and was therefore expected to give the greatest responses to nutrients.

THE RESPONSE TO FERTILIZERS IN THE POT EXPERIMENTS

The dry matter response by nodulated white clover following fertilizer application is dependent on the direct effect of the fertilizer on plant growth and its indirect effect on nodulation and nitrogen fixation. The effect of treatment is determined by the process that is most sensitive to the nutrient supplied. The effects that added nitrogen, phosphorus, potassium, calcium carbonate and magnesium have on growth, nodulation and

nitrogen fixation by white clover are discussed.

Nitrogen

Nitrogen (0-80 kg N/ha), as calcium nitrate, had little effect on yield of white clover. Haystead and Marriott (1979b) did a similar pot experiment with the Lephinmore deep peat but used ammonium sulphate as the source of nitrogen. They found that 0 to 120 kg N/ha had no effect on the yield of white clover and measurement of nitrogenase activity (by acetylene reduction) showed that nitrogen fixation was progressively reduced by increasing levels of added nitrogen and was replaced by uptake from the soil. Despite the different forms of nitrogen used the results from the two experiments suggest the effects were the same.

The lower concentration of nitrogen in the plants given 80 kg N/ha (2.27% N) than in those unfertilized with nitrogen (2.68% N) at harvest 2 in the Lephinmore peat is difficult to interpret but there are two possible explanations. It may be that the harvest was taken at the time when the plants which received nitrogen fertilizer were running short of nitrogen from the soil and had not fully developed the apparatus for nitrogen fixation. Legumes certainly go through a period of nitrogen starvation when changing from seed nitrogen to biologically fixed nitrogen (Day, 1972) and the same may be true when switching from fertilizer to fixed nitrogen. The different nitrogen concentrations had no effect on yield so the level of nitrogen in the shoot below which growth is limited must be less than 2.27%.

Secondly, the lower concentration of nitrogen in the shoots of plants given mineral nitrogen compared with those without nitrogen fertilizer, agrees with the observations of Haidock and Norris (1967) and Haystead et al. (1979) that effectively nodulated plants produce less dry matter per unit of nitrogen than do plants which rely on mineral nitrogen. One explanation for this is that the energy required for nodulation and nitrogen fixation in white clover is greater than for uptake and assimilation of mineral nitrogen.

Phosphorus and potassium

In the peats at the first harvest, and in the brown earth at the second harvest, there was a growth response to applied phosphorus and potassium. The results for the deep peat agree with the field experiments of Grennan and Mulqueen (1964a and b) in Ireland and Reith and Robertson (1971) in Scotland. However, neither group identified the interaction between the two elements. In Experiment 1, the response to one nutrient was dependent upon the level of application of the other. This synergistic effect agreed with Liebig's Law of the Minimum (Liebig, 1840) that plant growth is directly proportional to the supply of the nutrient present in the minimal amount. The chemical analysis of the shoot confirms that, in the deep peat at both harvests, and in the brown earth at the second harvest, plants were deficient in either phosphorus or potassium. Growth was

not increased when both phosphorus and potassium were deficient (< 0.2% P or < 0.9% K in shoot dry matter) when only one of the nutrients was applied. When the supply of either phosphorus or potassium limited growth, there was a response to application of the limiting element until the supply of the originally non-limiting element became inadequate and the concentration in the plant became deficient and reduced growth.

In Experiment 2, phosphorus deficiency (and to a lesser extent potassium deficiency) decreased the rate of leaf production and potassium deficiency increased the turnover of leaves and also, deficiency of both phosphorus and potassium decreased the area of a leaf but did not alter the weight. In general, the measured effects on growth agreed with the visual effects of deficiency described in Experiment 1.

Bouma and Dowling (1966) measured the effects of a range of concentrations of phosphorus and potassium in nutrient solution on the leaf area of subterranean clover and found that both elements reduced leaf area although the effect of phosphorus was greater than that of potassium except when severely deficient.

The effect of inadequate potassium on green leaf number was not primarily to reduce the rate at which leaves were produced but to accelerate senescence. This effect was expected because potassium is mobile in the plant and is in ionic form (Mengel and Kirkby, 1978). It must, therefore, be rapidly mobilised from older tissue and translocated to the meristem. Phosphorus

is also reputed to be a mobile element in the plant and Bieleski (1973) suggested a phosphate ion may make several circuits around the plant. However, movement must be slow relative to that of potassium and was not fast enough in phosphate deficient plants to maintain the same rate of leaf production of white clover sufficient in phosphate. Plants deficient in phosphorus looked more succulent than plants with sufficient phosphorus; although the degree of hydration of the leaves was not measured in the experiments it agreed with observations made by Atkinson (1973).

The overall effect of phosphorus and potassium was to greatly increase the leaf area of the plant and this, in turn, would lead to an increase in photosynthesis and the carbohydrate available for nitrogen fixation. In addition to effects on leaf area, these elements may have affected the rate of photosynthesis per unit leaf area. If the results for subterranean clover are at all applicable, the effect of potassium on photosynthesis may be purely on leaf number and leaf area because Bouma et al. (1979) found that photosynthesis per unit leaf area on all but the most severely deficient plants was marginal. Phosphorus, on the other hand, can increase photosynthesis per unit leaf area as well as increasing the leaf area itself (Bouma,1967).

The effect of level of added phosphorus on the number of root nodules was not examined. Experiment 9 in Part II of this thesis shows that 40 kg P/ha restricts nodulation and nitrogen fixation (measured by C_2H_2

reduction) compared to addition of 160 kg P/ha. It is well known that nodulation of legumes is restricted when phosphorus is inadequate but it is not clear whether there is a direct effect on nodulation or an indirect effect through improved nutrition of the host (see Andrew, 1977; Munns, 1977a). Phosphorus increased the number and weight of nodules and rate of nitrogen fixation (measured by acetylene reduction) in white clover (Crush, 1974; Gibson et al., 1975) and nitrogen fixation began earlier in Stylosanthes when the supply of phosphorus was adequate (Gates, 1974).

It is also a common observation that the concentration of nitrogen in the shoot continues to rise as the level of phosphorus increases after the dry matter response has tailed off (Andrew, 1977; Munns, 1977a) and so the quality of herbage, both in the nitrogen and phosphorus content, can increase with applications of phosphorus greater than those required for maximum growth. In Experiment 1 the concentration of nitrogen in the shoot rose with increased levels of added phosphorus even though in the deep peat there was also a marked growth response.

Few workers have investigated the effect of potassium on nodulation and nitrogen fixation. Mengel et al. (1974) and Feigenbaum and Mengel (1979), working with Vicia faba and Medicago sativa found that potassium increased nodulation and nitrogen fixation and, when plants were grown in nutrient solutions, the effect of potassium was probably related to the improved carbohydrate nutrition and

movement of assimilates in the plant. In Experiment 6, but not in Experiment 1, potassium decreased the nitrogen concentration in the plant and this decrease may have been dilution due to increased growth as suggested by Andrew (1977) for tropical legumes. On the other hand, in Experiment 6 the concentration of nodules on clover roots (number per gram of root) decreased as the level of added potassium increased. It may be that the high levels of potassium in the soil were slightly inhibitory to nodulation although no corroborative evidence can be found from the literature. However, the size of the nodules was not measured but it is thought that a few large nodules near the crown of the plant may be more efficient at nitrogen fixation than many smaller ones (Newbould and Haystead, 1978).

Magnesium

There was no response to added magnesium in either of the soils in Experiment 5 but, in Experiment 6 in the deep peat, there was a small response to 36 kg Mg/ha at harvest 2 and to 143 kg Mg/ha at harvest 3, with added potassium from 76-152 kg K/ha and calcium carbonate from 1450-5800 kg/ha. Therefore, for establishment and early growth, the soil contained sufficient magnesium and this agrees with Reith's suggestion that plants grown in soil with 3 mg Mg/100 g or more were unlikely to respond to magnesium fertilizer (Reith, 1963). Following removal of the herbage, and hence magnesium, at harvest 1,

in Experiment 6 there was a response to magnesium where growth was greatest at the following two harvests. If there were any antagonisms in uptake after calcium and potassium were applied, they did not affect growth.

There was a small increase in the number of root nodules in response to added magnesium and this may have been a direct effect on the growth of rhizobia because magnesium is known to be essential for growth (Vincent, 1962).

Calcium carbonate

The response by the plant to added calcium carbonate in Experiment 6 varied with harvest and was confounded by effects on nodulation at the lower levels (0-1450 kg $CaCO_3$/ha) and by possible effects on phosphorus uptake at the higher levels (2900-5800 kg $CaCO_3$/ha). The effects of low application of $CaCO_3$ will be discussed first.

Calcium carbonate both reduced acidity and increased the available calcium level of the soil. The other effects of lime can vary according to the type of soil and the more important effects include a reduction in the solubility of aluminium and manganese and a change in the availability of some trace elements in the soil. The effect of lime on clover growth was investigated only in the deep peat where levels of aluminium and manganese in the unlimed soil were low and unlikely to adversely affect growth and where trace elements were added in solution. In consequence, the effects of calcium carbonate on the

growth of clover in the deep peat can be confined to the effects of calcium and H^+ ions.

The growth of clover in the absence of calcium carbonate in Experiment 6 was probably most limited by nitrogen at harvests 2 and 3 because there were no root nodules. At harvest 1, and in Experiment 7 with tricalcium orthophosphate, the seedlings established and the level of nitrogen in the shoots was adequate (3.4% N) - presumably supplied from the soil because the roots had no nodules - and growth was restricted by the supply of calcium.

Where monocalcium orthophosphate was the source of phosphorus, in the absence of lime in Experiment 7 seedlings failed to establish, in contrast to the well established seedlings given tricalcium orthophosphate. This was probably because they lacked calcium as very little was stored in the seed and there was three times less calcium supplied in the more soluble salt. The calcium content of the seed in Experiment 7 was low compared to the other major nutrients. Nutrients mobile in the plant are translocated from the leaves to the seed but calcium is absorbed directly from the soil and is transported via the xylem to the seed. Therefore, the concentration in the seed depends upon the amount in the soil and the rate of transpiration (Marshner, 1974) and can vary depending on the source of the seed. The batch used in the experiments had a low calcium level in comparison to the other elements.

Root growth was restricted with $Ca_3(PO_4)_2$ in the absence of $CaCO_3$ in Experiment 7, where the roots were brown and were short, thick and profusely branched. Similar symptoms were described by Andrew (1960) in calcium deficient plants.

The effects of calcium and lime on the growth of rhizobia and on nodulation and nitrogen fixation, have been extensively studied, particularly the acid sensitive partnership of Rhizobium meliloti and Lucerne (Medicago sativa) but T. repens has not attracted much interest. Munns (1968, 1970), working with Lucerne, determined that the stage in nodulation most sensitive to acidity coincided with the curling of the root hair during infection and other workers have shown that nodulation in Pisum sativum and T. subterraneum is acid sensitive at the same stage (Lie, 1969; Lowther and Loneragan, 1968). Calcium and H^+ interact in infection and nodulation (see Munns, 1977b). O'Toole and Masterson (1968) applied calcium to a blanket bog (pH 4.2) in Ireland as either calcium sulphate or calcium carbonate. The sulphate did not alter the pH and required 4032 kg Ca/ha for white clover plants to nodulate whereas as little as 448 kg Ca/ha as calcium carbonate (1.1 tonnes $CaCO_3$/ha) raised the pH to 4.7 and resulted in similar nodulation. In the pot experiments 6 and 7 nodulation was suppressed in the absence of calcium carbonate (pH 3.8). On the other hand, in Experiment 7 most nodules were found on plants that received 2900 kg $CaCO_3$/ha (pH between 4.5-5.5) but more nodules greater than 1 mm long

occurred on plants given 1450 kg $CaCO_3$/ha (pH between 4.0-4.9).

These results support the data of Andrew and Norris (1961) and Munns and Fox (1977) that the number of nodules on the roots of white clover increased with the level of lime applied but the weight of nodular tissue either increased to a lesser extent or was unaltered. The important question is which type of nodulation results in the most nitrogen fixation and the results suggest that, if nitrogen fixation is dependent only on nodulation, it should be greatest where more of the larger nodules occur. However, nitrogen fixation itself is also thought to be sensitive to acidity (Munns, 1977b) and therefore maximal rates of fixation will be a result of the interaction between the two processes.

It is difficult to give a precise pH for optimum nodulation because the pH of the peat varied from the beginning to the end of the experiment. With 1450 kg $CaCO_3$/ha, the pH dropped from 4.9 to 4.0, and with 2900 kg $CaCO_3$/ha it dropped from 5.5 to 4.5. After liming, decomposition of the peat (Buckman and Brady, 1969) and nitrogen fixation by the plant (Raven and Smith, 1976) could result in H^+ production which would account for the decrease in the pH of the soil.

For growth, rhizobia are known to have a specific requirement for both calcium and magnesium and a requirement for non-specific divalent cations which is greater than that of both calcium and magnesium (Vincent, 1962).

However, application of up to 143 kg/ha magnesium did not substitute for calcium in obtaining nodulation in Experiment 6 (there were no Ca x Mg interactions).

The depression in yield when calcium carbonate was applied in large amounts in the pot experiments 6 and 7 agrees with similar effects observed by other workers in the field, e.g. when white clover was grown on a raised bog in Stirlingshire (Reith and Robertson, 1971) and in New Zealand on a gravel, thinly covered by loess (Adams and Lowther, 1970). In the latter example, application of phosphorus overcame the depression caused by high lime (Adams and Lowther, 1970).

In Experiment 6, the depression in yield with high lime may have been caused by interference in phosphorus nutrition, lime induced deficiency of zinc, boron or manganese, or it simply was the effect of a high salt concentration on the osmotic potential of the root. The most likely effect seemed to involve phosphorus nutrition because the concentration of phosphorus in the shoot decreased as the level of calcium carbonate increased. The salt effect was unlikely because calcium carbonate is not very soluble (1.4×10^{3} g/100 cc cold water) and because applications of much more soluble salts, KCl and $MgSO_4 7H_2O$ did not aggravate the effect when yield was depressed with high levels of added lime.

When phosphorus was applied as tricalcium orthophosphate, as in Experiment 6, application of lime could reduce the uptake of phosphorus by several related

processes: firstly, calcium from lime would inhibit the solubility of the sparingly soluble tricalcium orthophosphate and, secondly, with increasing soil pH, the proportion of $H_2PO_4^-$ (possibly the only form of phosphorus actively absorbed by plants (Hai and Laudelout (1966)) in relation to HPO_4^{2-} would decrease. At about pH 4.9, where in this experiment at harvest 1 the maximum yield was obtained, theoretically most of the phosphate in solution would be in the $H_2PO_4^-$ form, but at pH 7.0, where least growth occurred theoretically, there would be about equal quantities of $H_2PO_4^-$ and HPO_4^{2-} ions (Vogel, 1961). The reduction in pH as the experiment progressed would result in a corresponding change in the ratio of $H_2PO_4^-$:HPO_4^{2-} ions in the soil solution and would explain the observed shift in the maximum yield, which occurred at harvest 1 with 2450 kg (pH approx. 4.9) $CaCO_3$/ha, at harvest 2 with 2900 kg $CaCO_3$/ha and at harvest 3 with 5800 kg $CaCO_3$/ha (pH 5.5).

To test the hypothesis, application of tricalcium phosphate was compared with application of a more soluble salt, monocalcium phosphate. If correct, the latter salt should have increased growth compared to the less soluble salt at the greater levels of applied calcium carbonate. The experimental results supported the hypothesis because there was a significant increase ($P = 0.001$) in growth with 5800 kg $CaCO_3$/ha when the more soluble salt was applied but there was little

difference between salts with 1450 kg $CaCO_3$/ha. However, the depression in growth at high lime was not completely overcome with monocalcium phosphate possibly because Ca^{2+} ions in solution retard dissolution of all forms of calcium phosphate or there may be induced trace element deficiencies in addition to the effect of high lime on phosphorus supply.

A further explanation for the effect of lime on the uptake of phosphate by white clover may be taken from the work of Dunlop and Bowling (1978b) who measured the effect of pH on the electrogenic phosphate pump in the roots of white clover. For phosphate ions to enter cells which are negatively charged there must be active uptake. Measurements were made for short periods on plants that were 6 weeks old. The pump operated in solutions between pH 3.5 and 8.0 with an optimum at 4.3. If the pH of the peat at the beginning of Experiment 6 is considered, maximum yield at harvest 1 occurred with 1450 kg $CaCO_3$/ha (2.3 g/pot) at a starting pH of 4.9. Minimum yield (0.7 g/pot) occurred with 5800 kg $CaCO_3$/ha at a starting pH of 7.0. The minimum yield was about one third of the maximum and the data of Dunlop and Bowling (1978b) show that uptake of phosphate by the pump at pH 7.0 is about half that at pH 4.9. Therefore, sensitivity to pH of the electrogenic phosphate pump in the roots of white clover could explain why lime depressed yield in Experiments 6 and 7.

Therefore, while the mechanism of the depression is uncertain, the evidence suggests that lime interferes with phosphate nutrition and the alternative explanations (trace elements and salt concentration) are only of minor importance.

RESPONSES TO FERTILIZERS IN THE FIELD EXPERIMENTS

Fertilizers were applied to established ryegrass/white clover swards in the field experiments, by contrast to the pot experiments where fertilizers were applied at establishment.

Experiments 3 and 4 confirm that both phosphorus and potassium are required for continued growth of white clover in established pastures on peat whether cut or grazed (see also Grennan and Mulqueen, 1964a and b; Reith and Robertson, 1971; Reith et al., 1973; Floate et al., 1980) and are probably the major determinants of growth on a limed peat because white clover in Experiment 3 did not respond to the other elements applied. The experiments also suggest that the balance between fertilizers, for most efficient use, is as important in the field as it is in the laboratory. Chestnutt and Lowe (1970) cite several studies in lowland soils where there was a poor response by white clover to potassium when soils were low in available phosphorus.

In the pot experiments, where growth was enhanced by added phosphorus, there was an increase in the uptake of potassium and vice versa. Similar effects in the

field would retain potassium fertilizer against leaching and phosphorus fertilizer against loss of availability over time (Barrow and Shaw, 1975). Increases in growth would not therefore result in a corresponding dilution of phosphorus and decline in quality although some dilution would occur.

In Experiment 4, on the cut sward, white clover did not respond to phosphorus greater than 30 kg P/ha but the pasture had received 40 kg P/ha for 3 years before the fertilizer trial was set up and there may have been residual effects on growth because it is well known that effects of phosphorus fertilizer last for several years (Cooke, 1965). By contrast, there was a response to potassium up to 150 kg K/ha or more, despite the fact that 60 kg K/ha had been applied on each of the 3 previous years. It is possible that some of the applied potassium was leached away, e.g. O'Toole (1977) found that 8.6% of potassium fertilizer (given 112 kg K/ha) was leached from a blanket peat during the 4 weeks after application with 237 mm rain; and that potassium was taken up in luxury amounts early in the season so that at the first cut a substantial amount of potassium was removed from the system. Neither of these factors was measured but could have contributed to the large response to potassium. For cut swards, Brown (1957) and Reith et al. (1973) suggested that potassium be applied as small frequent dressings preferably after each cut to avoid luxury uptake and the possible depression of calcium and

magnesium uptake by potassium. There is little doubt that small frequent dressings make the most biologically efficient use of potassium fertilizer but for the low cost systems of hill farming they may not be the most economically efficient means of applying the fertilizer.

In contrast, most improved hill pastures on peat are grazed and much of the potassium in the herbage is recycled through the animal. Maintenance fertilizers are rarely required for grazed pasture on most mineral soils (Cowie, 1951) and, on peats, requirements are lower than for cut pastures (Grennan and O'Toole, 1966). On peats a single dressing of fertilizer in the spring soon after the pasture has begun growth (to minimise losses from leaching) should be sufficient to support the pasture as the potassium may be recycled three or four times throughout the season depending on the number of times the pasture is grazed.

THE VALUE OF POT EXPERIMENTS FOR PREDICTION OF RESPONSES IN THE FIELD

The differences between conditions of growth of white clover during establishment in the pot experiments described in this thesis and in the field fall into three categories: those deleted to reduce the complexity, those non-reproducible in the laboratory and those imposed by the experimental technique. Taking each category in turn the more important differences between laboratory and field will be discussed.

Factors which reduce complexity

In the field, clover grows in a pasture with a grass of high quality for animal nutrition (usually perennial ryegrass) and with indigenous species, and the greatest advantage in yield is derived when the total yield of the pasture is composed of about 40-50% white clover (Martin, 1960). Clover has a greater requirement for nutrients than ryegrass and disappears from pastures given low levels of fertilizer where ryegrass survives (Grennan and Mulqueen, 1964b; Reith et al., 1973), and ryegrass is a better competitor for nutrients (Chestnutt and Lowe, 1970). The reasons are related to differences between the species in root morphology, functional nutrient requirement, rates of uptake and the requirements for biological nitrogen fixation in clover (Loneragan and Snowball, 1969; Crush, 1974; Andrew and Johansen, 1978; Hall, 1978a; Robson and Loneragan, 1978; Dunlop et al., 1979). Therefore the fertilizer requirements for survival and growth in a pasture may be greater than when grown in monoculture.

In mixed pastures, neighbouring plant species may affect the root surface micro-organisms (Christie et al., 1978) and there may be effects of allelopathic chemicals on germination and seedling establishment (Rice, 1979), e.g. leachates from the leaves of three species indigenous to hill pasture, Festuca rubra, Pteridium aquilinum and Eriophorum vaginatum were found to reduce root length of germinating seedlings of white clover (Rogers and Bruce,1977).

A second simplification made in pot experiments is to mix finely ground fertilizer throughout the soil, e.g. a decrease in the pH of the soil in the field with depth may result in differences in the lime required for satisfactory nodulation in pot and field. In the field, particularly in wetter soils where cultivation is not possible, fertilizers are broadcast on the surface in the form of granules (superphosphate is generally sold in this form). Dissolution of fertilizer granules is complex and roots may be damaged or may proliferate around them (Drew, 1975).

Phosphorus is often applied as a split dressing, about half as superphosphate to aid establishment, and the rest, until recently, was applied as basic slag for benefit in later years (see Table 1: Newbould, 1974/5). Because basic slag is no longer available, ground mineral phosphate may be suitable as a substitute. In all the experiments except one phosphorus was applied in one form, as tricalcium phosphate.

Factors which cannot be reproduced in the laboratory

The major factor in this category is the climate. The climate in the glasshouse is very different from that in the field. Temperatures are generally higher and fluctuations are greater and the quality of light which has passed through glass or which comes from lamps is different from outdoors. Experiments conducted in growth rooms give a greater control of light temperature and humidity but the gradual diurnal and seasonal changes are not easily reproduced, nor are the effects of

micro-climate in either the glasshouse or the growth chamber.

Temperature affects mineralisation and movement of nutrients in the soil, uptake of nutrients by the plant and growth (Simpson, 1965; Sutcliffe, 1977; Nye and Tinker, 1977; Vernon, 1978) and it is the major climatic restraint to growth on the hills (Newbould, 1979). In the pot experiments the temperature in the greenhouse was held at 13^{0}C or above and the growth rooms were set at 15^{0}C day/10^{0}C night whereas the air temperature is greater than 13^{0}C at Lephinmore only from mid May to mid September and, at Sourhope, from mid April to mid October. The soil temperature (5 cm) reaches 13^{0}C at Sourhope from mid May to late September but at Lephinmore this temperature is reached only for a few days in the year.

Rainfall can affect the amount of fertilizer leached from the soil and the rate of dissolution of the chemicals. If rainfall is high, and temperatures lower than those required for germination and growth occur after a pasture is sown, fertilizers (particularly nitrate-nitrogen) may be leached away. In the pot experiments a saucer placed beneath each pot held excess water and prevented leaching.

The germination and survival of seedlings in pasture in the field by the end of the establishment year often can be less than 10% (Charles, 1961). In pot experiments, more than 90% of seeds germinated and survived the experiment and it may be that the genotype of plants in pot experiments are different to those in the field

and respond differently to fertilizers because plants which are tolerant of adverse climates generally yield less (Snaydon and Bradshaw, 1962a). This is true of cultivars of white clover as those bred for tropical pastures, e.g. Ladino, yield more than those bred for temperate pastures, e.g. Blanca, which yield more than those bred for hill pasture, e.g. S184.

Factors imposed by the experimental techniques

Factors in this category refer to the techniques used in pot experiments. The soils collected from the field were dried to a low enough moisture content to allow fertilizers to be mixed into the soil (using a food mixer) without caking. To avoid undue death of the micro-organisms, the mineral soils were not air dried, as is often done after collection from the field. The peats could not be air dried because rewetting is very difficult. However, storage of damp soil may have meant that the availability of nutrients in the soil could have changed with time.

The mineral soil was sieved and the peats were shredded before fertilizers were mixed with them and, as the mineral soils are usually cultivated in the field but the peats are not, the techniques applied to the peats were further removed from the field than those applied to the brown earth.

Pots were watered from the bottom once seedlings were established by filling the saucers below the pots

with water once or twice each day. (For about 2 weeks after germination the seedlings were sprayed with water). In the very hot summer of 1975, the plants grown in the Glensaugh peat in Experiment 1 were watered almost continuously. The supply of water in the three soils in the field would have been very different from each other and from that given in the pot experiments. Evaporation from the surface of pots can result in an extremely high concentration of nutrients at the soil surface and can damage plants, particularly seedlings.

Root growth in pot experiments is restricted by the size of the pot and the root system tends to be more branched than in the field (Powell and Daniel, 1978).

In the pot experiments a yield of 7 g/pot is equivalent to approximately 10,000 kg/ha. The maximum yield possible from a clover sward in temperate regions was calculated to be 9000 kg/ha by Chestnutt and Lowe (1970) because of constraints to growth from the optimum leaf area index (of approximately 4) and the length of the growing season. It is not possible to quote yields for pure white clover swards from the field during the year of establishment but a series of field trials aimed at measuring the effect of inoculation of white clover seed with rhizobia (Newbould et al., 1980) on the three soils used in Experiment 1, where clover was grown without ryegrass, may be used for comparison:

Soil		Pot H1	Pot H2	Pot H1 + 2	Field (Newbould et al., 1980)
		Yield (tonnes/ha)			
Sourhope brown earth	range	2.9-5.0	4.7-9.7	7.7-14.7	0.4-2.6
	mean	3.9	7.0	10.9	1.5
Glensaugh dry peat	range	1.7-6.1	1.9-20.3	3.6-26.4	- -
	mean	3.6	9.6	13.2	0.13
Lephinmore deep peat	range	0.4-7.9	1.3-10.6	1.7-18.5	- -
	mean	3.1	3.9	7.0	0.04

Yields from the pot experiments were much greater than those expected from the field but in the pot there can be a pronounced edge effect with the plant growing over the sides of the pot and effectively increasing the irradiated area of leaf. The response to fertilizers at harvest 1 may be taken as the most applicable to the establishment year.

It is of interest to note that the lowest yields of clover were in the order expected from yields of improved pasture in the field: brown earth > dry peat > deep peat. The maximum yields were in a different order: dry peat > deep peat > brown earth and the order could be related to the time of year the plants in the experiments were grown; the dry peat experiment took place in mid summer with the deep peat and brown earth experiments in spring. The growth period in the brown earth experiments was 2 weeks prior to the deep peat experiment.

Therefore, the pot experiments are a relatively cheap and easy way to screen, from a wide range of levels of application of nutrient, those most likely to be of value

for trial in the field. They are of little use to predict absolute values of fertilizer to apply in agricultural practice but they do show the relative magnitudes of response to fertilizer elements, the likely interactions between elements and can also identify concentrations of nutrient in plant tissues below which growth is limited by deficiency of the element and above which growth is limited by toxicity of the element. Further, the cause of problems encountered in the field can be investigated in the laboratory where conditions are controlled, although such investigations are not described here.

Toxic levels of nutrient in the plant may cause depressions in plant growth in the field more often than the pot experiments have suggested. The response to fertilizer will depend on climate. Where the response to fertilizer is limited by climate, concentrations of nutrient may build up to toxic levels in the plant through luxury uptake (see Fig. 2). The likelihood of this in the field depends on whether fertilizers are applied for maximum responses in years when the climate is most favourable for growth, unfavourable for growth, or aimed for optimum growth, say, in 8 out of 10 years.

THE CONCENTRATION OF THE NUTRIENT IN THE SHOOT IN RELATION TO YIELD

There are three concentrations of a nutrient in the plant that are useful in the diagnosis of toxicity or deficiency in plant nutrition. They are the concentration in the shoot below which growth is limited by an

element, often called the critical concentration, the concentration at which deficiency symptoms appear and the concentration above which the element is toxic to growth. In grazed pasture, another value is important: the concentration which satisfied the mineral requirements of the animal.

The critical concentration of a nutrient proposed by Ulrich (1952) has been criticised by Loneragan and Snowball (1969) because it does not take into account the differences in mobility of elements in the plant. The concentration of non-mobile elements, e.g. calcium, in mature leaves may be very much higher than the concentration of the non-mobile element in the young leaves and at the meristem. Therefore, if the concentration of a non-mobile element in the growth media fluctuates, nutrient analysis of the whole shoot would either give a false level for the minimum concentration in the tissue necessary for maximum growth or indicate no deficiency of the element. Loneragan and Snowball (1969) proposed 'the functional nutrient concentration' which is 'the minimal concentration of nutrient within the plant organism which can sustain its metabolic functions at rates which do not limit growth'. For elements which are very mobile in the plant tissues, the functional nutrient requirements would be similar to the critical concentration, e.g. nitrogen , phosphorus, potassium.

The 'functional nutrient concentration' is of great value in physiological studies of nutrient requirements

between species but, in the field, concentrations of non-mobile nutrients, like calcium in the soil, are unlikely to fluctuate markedly during the growing season, and levels of the nutrient in the whole shoot of plants grown under such constant conditions of nutrient supply would give a good indication of the non-mobile nutrient status in the plant.

Therefore, while there are other drawbacks related to critical concentrations in addition to the criticism above, e.g. the value can vary somewhat with the age of the plant and stage of maturity (McNaught, 1958) and care must be taken to stipulate the age and part of the plant, they can be a useful diagnostic character of deficiency or toxicity in the field.

The concentrations of phosphorus, potassium, magnesium and calcium in the experimental plants will be discussed as outlined above.

Phosphorus. The concentration of phosphorus in the shoot varied from 0.08 to 0.46 in Experiment 1, a similar range to that reported in a survey by Spedding and Diekmahns (1972). The concentration in the shoot (leaf + petiole) below which plant growth was limited was about 0.20% P which level is in close agreement with values of 0.20 to 0.25% P from other studies (Andrew, 1960; McNaught and During, 1970; Jackman and Mouat, 1972). Levels of nutrient in the shoot at which deficiency symptoms occur and where the nutrient is toxic to growth were not determined because deficiency symptoms were not obvious and

levels of nutrient in the shoot in the experiment were never toxic.

The phosphorus content of herbage required by sheep in a grazed pasture is about 0.32% P in dry matter (Swift, 1972).

Potassium. In Experiments 1 and 6, where potassium treatments were applied, the concentrations of potassium in the shoot ranged from 0.27 to 4.01%, a wider variation than those reported by Spedding and Diekmahns (1972) of 1.54 to 3.8. Plant growth was limited by potassium when the concentration in the tissues was less than 0.9% (Experiment 1), although harvest 2 in Experiment 6 suggests the value may be nearer 2.0% K. Similar contradictions occur in the literature. McNaught (1958) found 1.8% K to be the critical concentration, while Andrew (1960) found it to be 1.1% K. In all the studies the material analysed was mature leaf and petiole.

Deficiency symptoms occur at about 0.8% K in shoots and there is some evidence that a level of potassium in the shoot greater than 4.0% is toxic. This is important for a nutrient which is reputed to be taken up in luxury amounts.

Greater levels of potassium are required for plant growth than for animal health (Allaway, 1975) and much of the potassium in herbage is recycled in the urine (Floate et al., 1980). There is a danger from hypomagnesaemia in animals if the potassium content is greater than about 3% and magnesium is less than 0.2% dry matter (Wolton, 1963).

Calcium. The level of calcium in the shoots ranged from 0.9 to 3.8% in Experiment 6. In this experiment, levels of calcium in shoots less than 2% occurred in the absence of calcium carbonate. In practice, lime applied to decrease acidity for adequate nodulation and nitrogen fixation and to reduce competition from indigenous species, will provide sufficient calcium for plant growth. Bearing in mind the criticisms made by Snowball and Loneragan (1969), it is suggested that, where concentrations of calcium are less than 1% in the shoot, plant growth would be limited by the supply of calcium. This figure agrees with that taken from the data of Andrew and Norris (1961) (see Introduction, p.38). Levels of calcium which result in deficiency symptoms or which were toxic to plant growth were not determined.

The requirement by ewes for calcium in herbage is about 0.6% which is less than half the normal level in clover (Swift, 1972). However, grasses often contain about one third less the concentration of calcium than clover (Spedding and Diekmahns, 1972) and levels greater than 0.6% Ca in clover in a ryegrass/white clover pasture are probably needed to sustain the animal.

Magnesium. Magnesium levels in the plant ranged from 0.07 to 1.44% in Experiment 6, a somewhat wider range than that reported by Spedding and Diekmahns (1972) of 0.15 to 0.29% Mg. There was a dry matter response from plants with 0.29% or less magnesium in the shoot, a value which disagrees with McNaught and Dorofaeff (1965). The greatest concentration recorded was 1.44% Mg in the dry matter, this level not being toxic to growth.

In pasture, 0.06% Mg will satisfy animal mineral requirements (Swift, 1972) but to prevent hypomagnesaemia levels should be greater than 0.2% in dry matter when levels of potassium are greater than 3% (Wolton, 1963).

Luxury uptake. Luxury uptake of nutrients is usually associated with potassium (Whitehead, 1966; Buckman and Brady, 1969; Spedding and Diekmahns, 1972). However, if levels of nutrient in the tissue are greater than the functional nutrient requirement or the critical concentration for elements which are mobile in the plant, they may be considered as being taken up in luxury amounts. The critical concentrations found in the present experiments of phosphorus, potassium and magnesium were 0.20, 0.9 and 0.29% dry matter respectively. Where large amounts of phosphorus, potassium and magnesium were applied in Experiments 1 and 6, levels in the plant reached 0.46% P, 4.01% K and 1.4% Mg and, therefore, the three elements were taken up in luxury amounts. The functional requirement for calcium was not determined in this study, but Loneragan and Snowball (1969) suggested it lies between 0.1 to 0.2% in most legumes. Levels of calcium in the shoots of white clover usually range between 1 and 3%, such levels representing a considerable amount of luxury uptake.

THE EFFECT OF AN APPLIED NUTRIENT ON THE CONCENTRATION OF OTHER NUTRIENTS IN THE SHOOT

In the experiments, applications of a range of a fertilizer element resulted in a range of concentrations

of that element within the plant. The effect of one applied nutrient on the concentration of other nutrients will be considered in this section.

According to Mengel and Kirkby (1978) the concentration of cations in a plant is fairly constant regardless of the type, and absorption of cations is a non-specific attraction to the negatively charged cell. Therefore, an increase in the ratio of one type of cation in the nutrient media will result in a greater uptake of that type and a lower uptake of other types of cation. Potassium, however, may be taken up actively (Ansari and Bowling, 1972) and over a greater length of root than the two other major nutrient cations, calcium and magnesium (Russel and Clarkson, 1976) and is often, therefore, the more successful competitor for uptake. However, the major nutrient cations, potassium, calcium and magnesium cannot substitute for each other in function. Therefore, if uptake of one cation A results in a lowering of the concentration of another cation B in the tissues below the critical concentration of the latter, growth will be impaired. This imbalance in mineral composition which inhibits growth is equivalent to the 'toxic' range of a nutrient when the concentration of cation A is plotted against yield (see Fig. 2, p.33).

In pasture species, the balance between nutrients is also important in animal health, particularly in the prevention of hypomagnesaemia as discussed above and for maintenance of the calcium content (Van der Kley, 1957).

Increases in the absorption of anions result in a greater negative charge in the cell and may in turn result in a greater attraction and uptake of cations.

Workers who have conducted fertilizer trials with white clover or grass/white clover pastures in the field have reported interactions between cations when the total herbage was analysed but few workers have separated the species for analysis. The results from total herbage analysis may be misleading because deficiency of a nutrient in white clover may reduce nitrogen fixation and lead to nitrogen deficiency in grasses with an accumulation of the nutrient which limits the growth of clover (McNaught, 1970). The absolute levels of fertilizer at which interactions occur and affect yield will depend mainly upon the soil type and rate of growth of the plant. For a given level of fertilizer of, say, potassium chloride, the indigenous levels of all cations in the soil and the cation exchange properties of the soil would influence the relative concentrations of the cations in the soil available for absorption. Similarly, for a given range of fertilizer, a rapid rate of growth caused by, for example, high temperatures in the greenhouse, would dilute nutrients in the tissues and interactions at the lower range of concentration would be more evident than in the field. Where low temperatures limit the rate of growth, concentrations of nutrient in the tissues may build up and toxic effects at the higher range of a nutrient may be more important.

The interactions evident from the experimental work described here resulted from:-

(a) The effect of applied phosphorus. Phosphorus concentrations from 0.08 to 0.46% P in the shoot dry matter were the result of an application of added phosphorus fertilizer in Experiment 1. From 0.07 to 0.20% P in the dry matter the concentration of calcium in the tissue increased when plants were grown in the Lephinmore peat. Plants grown in the Sourhope soil had few concentrations of phosphorus within this range but there was a similar trend. There was little relationship between the concentration of phosphorus above 0.2% in the dry matter and the concentration of calcium or between any concentration of phosphorus and magnesium in the plant in either soil. Andrew (1960) applied monosodium orthophosphate to a humic gley and found that phosphorus had no effect on the levels of calcium and magnesium in clover.

(b) The effect of applied potassium. In the Lephinmore peat, in Experiment 1, applications of potassium fertilizer resulted in a range of potassium concentrations in the shoot from 0.3 to 4.0% of the dry matter. When the concentration of potassium was below 2% there was an increase in the concentrations of calcium and there tended to be a similar relationship in the brown earth. Also in the peat, but not in the Sourhope brown earth, the concentration of magnesium increased when the concentration of potassium was less than 1%. The information

from Experiment 6 supports results from Experiment 1 that (1) below 2% K the concentration of calcium in the plant increases, (2) in the range from 2 to 4% there is little interaction, (3) below 1% K the shoot concentration of magnesium increases in the peat, and (4) between 1 to 4% K there is little effect on the concentration of magnesium. These results are very similar to those of Andrew (1960). Neither the concentration of calcium nor magnesium was depressed by potassium treatment below the critical concentration or below the optimum for animal health.

The relationship between phosphorus and potassium in Experiment 1 is more difficult to interpret as the concentration of both nutrients varied with fertilizer treatment. However, in Experiment 6, the concentration of phosphorus decreased as the concentration of potassium increased and the effect was most apparent below the critical concentration of potassium. This was probably because with each increase in concentration of potassium there was an increase in growth and a relative dilution of phosphorus in the tissues, although Andrew (1960) suggested that the chloride ion, added as potassium chloride, could have reduced the uptake of phosphorus.

(c) The effect of applied calcium. In Experiment 6, as the concentration of calcium increased in the plant, there was a somewhat similar decrease in the concentrations of potassium and magnesium, and again these results agree with Andrew (1960).

The decrease in the phosphorus concentration in the plant with increasing levels of calcium carbonate has been discussed above in relation to chemical interactions in the soil and may not therefore be related to antagonisms in root uptake.

(d) The effect of applied magnesium. With the levels applied in the experiments, magnesium had little effect on the concentration of other nutrients.

It may be concluded that interactions between two cations were strongest where the concentration of one of the nutrients was below the critical concentration. Under such circumstances, a restricted yield caused by lack of one cation A with an unaffected or greater uptake of the other cation B, would lead to an accumulation of cation B in the tissues. Where the concentrations of both cations were above the critical concentrations, there was little interaction between them except for a decrease in the concentration of calcium between 1 to 2% K in the tissues. Reith (1963) found that applications of potassium required to maintain yields had little effect on the magnesium content of crops. In the experiments described here, the concentrations of cations in the plant caused by fertilizer treatment were as high or greater than those reported by Spedding and Diekmahns (1972) from several studies of field grown white clover and were in many cases three times greater than the critical concentration of the nutrient in the plant but, even at such high levels, there were no strong antagonisms

between different types of cation.

Concentrations of nutrient in the shoot were toxic to the plant in the experiments on one occasion only when 320 kg K/ha were applied to the Sourhope brown earth in Experiment 1 at harvest 1. The toxicity was not caused by a depression in the magnesium or phosphorus concentration below the critical concentration, nor were the concentrations of calcium and nitrogen low.

Therefore, if the fertilizer management of the sward ensures that the concentrations of nutrients in the tissues are maintained a little above the critical concentrations, particularly the concentration of potassium, there should be no adverse effects of nutrient antagonisms.

THE VALUE OF LEAF ANALYSIS TO PREDICT REQUIREMENTS FOR MAINTENANCE FERTILIZER

Problems arise as to how to measure easily the need for these fertilizers and estimate the amounts to apply. The most reliable but costly and time consuming way of collecting data to advise the farmer of fertilizer requirements for a crop is to collect data from the field from trials carried out on different soils and in different environments over several seasons. Other less costly, quick and simple methods of analysis of soils or vegetation which predict fertilizer requirements accurately are required and the present advice for maintenance fertilization of grazed hill swards is to reapply lime and phosphorus every 3 to 5 years (WSAC, 1975; ESCA, 1977; NSCA, 1978; ADAS, 1979). In practice, however,

maintenance fertilizers are often only applied after the sward has deteriorated. Evidence suggests that white clover is more sensitive to the available nutrient content in the soil than the grasses (McNaught, 1958), so leaf analysis of white clover may be a valuable method to predict maintenance fertilizer requirements for pasture.

The experiments described in this thesis have measured the critical concentrations of nutrients in the shoots of white clover during the vegetative stage of growth. The critical concentrations of phosphorus and potassium were on the whole similar in the two soils used and agreed with those suggested by other workers for white clover grown in different environments. They could therefore provide a stable basis for prediction of fertilizer need, provided care is taken to standardise the part and stage of growth of the plant at sampling. Leaf analysis has been successfully used for identification of nutrient requirements in other perennial crops, particularly for tree crops (see Chapman, 1966), but has not been found to be generally useful in determining the quantity of fertilizer needed (Munns, 1977a; Mengel and Kirkby, 1978). However, McNaught (1970) has reported that the method has been used successfully in white clover pasture in New Zealand. Methods of soil analysis are no better and may be worse indicators of the requirement for fertilizers, as shown for phosphorus by Pimplaskar et al. (1980).

Therefore, studies are required to develop methods of analysis which accurately predict fertilizer requirements. Leaf analysis, if not used routinely by the advisory services, could be a very useful tool to expand knowledge of the soil's ability to retain applied fertilizer against leaching and fixation and to formulate maintenance fertilizer regimes for different soil types.

Other methods of assessing the nutrient status of plants, and in particular subterranean clover, by measuring the increase in yield or photosynthesis of a detached leaf after treatment with or without the element to be tested, have been tried. The method has been used successfully in plants deficient in phosphorus grown under laboratory conditions (Bouma and Dowling, 1976) but has not been quite so successful when plants were deficient in potassium and magnesium (Bouma et al., 1979). If techniques were developed to measure the major elements, difficulties could be envisaged when leaves need to be transported from the field to the laboratory for assay.

An interesting method of assessing the superphosphate requirements of crops and pasture in Western Australia is based on the 'Decide' computer model which attempts to reconstruct a fertilizer response curve for each farm or paddock. It takes into consideration the research workers' experience in assessing, e.g. the soil type, crop, method and time of fertilizer application together with the farmers' local knowledge and experience. Once the

response curve has been calculated, it can be used to calculate the most economic rate of application of fertilizer and strategy of application (Bennett and Bowden, 1976).

FUTURE WORK

The results of this investigation, while only preliminary, have identified the areas of nutrition which are important for the growth of white clover in improved hill pastures. The responses and interactions identified in this work can form the basis for a laboratory and field study of the fertilizers required for establishment of white clover and ryegrass in two or three of the main hill soil types. A series of experiments of this type would not only give practical information for revision of recommendations of fertilizer need but would also allow a comparison between pot and field so that the value of the pot experiments carried out in the present study can be assessed and, if found useful, further experiments on other soils can be used to greater advantage. At present, the relative importance of the differences between pot and field previously discussed are not known precisely.

There is a wide area of ignorance about the amount and frequency of maintenance fertilizers required, both in cut and grazed swards. Data from Experiment 3, where herbage had been grazed before fertilizer top dressings were applied, and from Floate et al. (1980) suggests that, contrary to expectation, grazed swards on peat require frequent dressings of potassium. Dressings of lime and

fertilizer, which are normally applied every 3 to 5 years, should be considered in the light of the balance required between phosphorus and potassium and the interaction between lime and potassium as indicated by Floate et al. (1980). The lack of dry matter response to nitrogen in contrast to the interaction between nitrogen fertilizer and the nitrogen content of the clover in the Lephinmore peat in Experiment 1 emphasises the importance of understanding the relationship between mineral and fixed nitrogen in the growth of white clover. For a detailed understanding of the mechanisms involved, knowledge of the amounts and pathways of transfer of nutrients, both above and below ground, and the losses through leaching is required. Such a study would necessitate investigations in both the field (grazed and cut) and in the laboratory (cut only), probably in boxes rather than in pots, and would require more sophisticated techniques than those used in the present investigation, e.g. to study the relationships between mineral and fixed nitrogen ^{15}N would have to be used. Once the method of leaf analysis has been assessed, it may be used to give a direct and continuous monitor of plant nutrient status.

Therefore, the information presented here, coupled with that suggested for collection, should help to make the establishment and maintenance of white clover and companion species on hill soils more predictable and reliable in the future, and possibly less costly if fertilizers are used more effectively.

PART II. THE EFFECT OF INOCULATION WITH MYCORRHIZAL FUNGI ON THE GROWTH OF WHITE CLOVER

INTRODUCTION

The review which follows examines some of the more recent and more relevant information which relates the possible benefits, in nutrient uptake and plant growth, of inoculation of white clover with mycorrhizal fungi to growth in improved hill pastures. It briefly describes how the fungi are classified, the morphology of the infection and the natural occurrence of mycorrhizal plants. There is discussion on the effect of mycorrhizas on plant growth, interactions with micro-organisms including Rhizobium, and the effect of the environment on the symbiosis. There is some comment on specificity between the fungus, the host and the soil type. The possible application of inoculation of agricultural crops with mycorrhizal fungi is considered in the final section with particular reference to inoculation of white clover in hill pastures.

TAXONOMY

Fungi that form vesicular-arbuscular (VA) mycorrhizas with plant roots are assigned to the order Mucorales and the family Endogonaceae. Alternative names for the VA mycorrhizas are endotrophic, phycomycetous, arbuscular, zygomycetous and endogonaceous mycorrhizas (Walker, 1979). The genera and species are recognised by the morphology of the chlamydospores or resting spores and have the largest known fungal spores, often 100-200 μm diameter, although much of the recent work stresses the importance

of the smaller spored species which have in the past been lost when soils were wet sieved through meshes generally greater than 100 μm (Hall, 1977a; Walker, 1979). The smallest spores may be those of Glomus tenuis at about 10-13 μm diameter.

There are two main systems of classification. In one, all fungi that form VA mycorrhizas are assigned to the genus Endogone and the spore types are given descriptive names, e.g. "yellow vacuolate", "honey coloured sessile" and "white reticulate" (Mosse and Bowen, 1968). The other system recognises seven genera of fungi within the Endogonaceae, four of which form VA mycorrhiza and they are Glomus, Gigaspora, Acaulospora and Sclerocystis. Members of the genus Endogone in this system do not form VA infections (Gerdemann and Trappe, 1974). Species are given a binomial. It is not always possible to cross-link species in the two systems, e.g. Glomus mosseae and "yellow vacuolate" describe the same fungus, and "honey coloured sessile" is the same as Acaulospora laevis but the spore types "white reticulate" and "bulbous vacuolate" have not been assigned a corresponding binomial (Hayman, 1978).

The four genera Glomus, Acaulospora, Gigaspora and Sclerocystis form morphologically distinct hyphae and infections (Gerdemann and Trappe, 1974) and can be identified by an experienced eye in the roots of plants grown in the greenhouse (Abbott and Robson, 1978). Work

by Abbott and Robson (1979) suggests that in pot cultures at least it may be possible to identify species of endophytes within plant roots.

THE INFECTION

The morphology of VA mycorrhizal infections is described by Mosse (1963), Nicolson (1967) and Gerdemann (1975) amongst others.

Infections arise from a germinated spore, an infected root segment or a piece of hyphae (Johnson, 1977; Powell, 1976b). The infecting hyphae produces an appressorium on the surface of the root from which the hyphae penetrate the plant root. The fungus colonises the root cortex (usually the mid cortex), but never infects the stele or root nodules formed by rhizobia and only rarely invades the root tip (Lanowska, 1966; Gerdemann, 1975). The mechanism by which the endophyte is excluded from these tissues is unknown although it has been suggested that high levels of phosphorus in the root nodules (two to three times more phosphorus per unit dry matter than the root on which they are formed) (Mosse, Powell and Hayman, 1976) may inhibit colonisation (Crush, 1974).

The hyphae run through the root cortex, parallel to the root axis; they may be intracellular or intercellular depending on the host species. Soon after infection, arbuscules are formed in some cortical cells. The nucleus of the cell enlarges and the volume of cytoplasm can increase 23 fold (Cox and Tinker, 1976), and

starch granules disappear. Hyphae in the cell branch repeatedly until the cytoplasm in the cell is filled with the structure and the ultimate branches of the arbuscule may be less than 1 μm diameter. The arbuscules are transient structures (Kaspari, 1973) estimated to be functional for 2-13 days (Bevege and Bowen, 1975) or 4-5 days (Cox and Tinker, 1976), after which they are probably digested by the host cell. They are thought to be the site of exchange of materials between the fungus and the host (Cox and Tinker, 1976) and are analogous with haustoria formed by pathogenic fungi (Gerdemann, 1975). After the arbuscule is destroyed, the nucleus of the host cell returns to normal size.

Vesicles generally occur in older parts of the infection. They are terminal or spherical structures that contain oil droplets and are temporary storage bodies which form in or between cortical cells in most species. Gigaspora spp. do not form vesicles in roots but form thin walled structures in the soil which probably function for storage but are not analogous to internal vesicles (Gerdemann, 1975). The walls of the internal vesicles may thicken and become reproductive structures (Gerdemann, 1975).

Infections do not normally damage or alter the morphology of the root but in rare cases vesicles protrude from and rupture the root (Mosse, 1963; Gerdemann, 1975). Roots of some hosts, notably maize,

onions and tomatoes, are bright yellow when infected but the colour fades when exposed to light (Daft and Nicolson, 1972).

A loose network of mycelium extends into the soil at least 1-2 cm from the surface of the root (Tinker, 1975a). The external hyphae is dimorphic; short fine hyphae (2-3 μm diameter) arise from knobs on the coarser, aseptate hyphae (in most species 20-30 μm diameter). The fine hyphae are thought to penetrate organic matter; they are ephemeral and become septate when they senesce (Nicolson, 1967). Resting spores are formed on the coarse external hyphae and they may or may not be borne in sporocarps (Nicolson, 1967).

Infections are sporadic and are scattered throughout the root system (Daft and Nicolson, 1972). The spread of infection within and between root systems generally occurs by reinfection from external hyphae and not by extension of the internal hyphae (Sanders et al., 1977).

OCCURRENCE

VA endophytes form mycorrhizas with angiosperms, gymnosperms, pteridophytes and bryophytes (Maeda, 1954; Cooper, 1973; Johnson, 1977). Some families of plants rarely form VA mycorrhizas and included in these are families whose members are commonly found in temperate regions such as Cruciferae, Chenopodiaceae, Caryophylaceae, Cyperaceae, Juncaceae,Urticaceae and Polygonaceae. Other plants which fall into this category are those which form

alternative types of mycorrhizal association such as the Ericales, the Orchidaceae, the Pinaceae and the Betulaceae. Smith (1974) described the different types of mycorrhizal association.

Plants infected with VA mycorrhizas are found in all climatic regions of the world and in all ecological niches except for plants growing in water (Mosse, 1973a; Hayman, 1978) and in severely disturbed sites (Moore, 1979).

Endophytes are non-specific in the sense that one endophyte will infect a wide variety of host species (Mosse, 1973a) but a fungus may have different effects on different hosts (see p.198).

The absence of plants which form VA mycorrhizas in the Ericales, the Cyperaceae, Juncaceae and the Cruciferae may affect the establishment of VA mycorrhizas in reseeded pasture. Plants of the Juncaceae and Cyperaceae, e.g. Eriophorum, Trichophorum, are predominant in blanket bog vegetation. The density of inoculum in such soils will be less than in soils where most of the species present form VA mycorrhizas, although there is a possibility that the inoculum density in wet sites may be greater than would first appear if the bryophyte, Sphagnum, which is also predominant in blanket bog vegetation, forms VA mycorrhizas. However, the wetness of the soil would discourage mycorrhizal formation so, on balance, the density of inoculum in a blanket bog will probably be low.

The vegetation on dryer peat soils is often dominated by Ericacous species, particularly Calluna vulgaris, which form mycorrhizas with a different group of fungi, the Ericaceous mycorrhizas (Smith, 1974). The density of VA mycorrhizal inoculum in this group of soils then would be low but, in addition, there is some evidence that formation of sheathing mycorrhizas is inhibited in soils which have grown heather (Forestry Commission Bulletin 36) and it may be that VA mycorrhizas are similarly affected.

It is a recommended practice to break down the surface mat of vegetation on unploughable hill land with a crop of turnips two years before pasture is reseeded (ADAS, 1978). Turnips are crucifers and do not form mycorrhizas and therefore the density of inoculum in the soil could be drastically reduced during the intercropping years by the death of hyphal and infected root segments, leaving only spores as inoculum.

The distribution of spores in soils has been surveyed in many parts of the world, e.g. Scotland and North America (Gerdemann and Nicolson, 1962, 1963); Australia (Mosse and Bowen, 1968; Abbott and Robson, 1977a); the Pacific North West, U.S.A. (Gerdemann and Trappe, 1974); and Pakistan (Saif and Iffatt, 1976). The only survey of hill soils in the world was carried out by Crush (1973) on tussock grassland in Otago Province, New Zealand, at altitudes 300-2,000 M above sea level. Endomycorrhizal spores and infections were ubiquitous to the grassland, and the most common spore types were from two species of

fungi, Acaulospora laevis, Glomus fasciculatus, and from the G. microcarpa-G. macrocarpa group. The number of spores in soils was extremely variable even from adjacent samples; most fell in the range of a few hundred to several thousand per litre of soil. Sparling and Tinker (1978a) found 600-2800 spores/litre soil from grassland sites in the Pennines. In addition, Glomus tenuis, the spores of which are very small and not normally included in spore counts, was common on the roots of grasses found at high altitudes. Levels of infection of plant roots were greater than 30% in three quarters of the plants surveyed and these results agree fairly well with levels of infection found in Pennine grasslands of about 50% (Sparling and Tinker, 1978a).

Some of the surveys have been accompanied by measurements of levels of infection in plants growing in the same soils but identification of the fungi in infections is difficult below the generic level. It is thought that many fungi do not sporulate in undisturbed soils such as those under pasture and forest. The high density of plant roots in undisturbed soils would allow the spread of the fungus without committing large quantities of metabolites into resting structures (Baylis, 1969; Crush, 1975). Hall (1977a) noted a lack of correlation between spore numbers and levels of infection in New Zealand bush soils. Because VA mycorrhizal fungi are obligate symbionts, the fungi which form infections cannot be grown on synthetic media and identified when

spores are formed. Therefore many fungi that are infective in the field may be missed in spore surveys.

Glomus tenuis is probably the most common fungus that infects plants in hill pastures (Crush, 1973; Sparling and Tinker, 1975) and may be the species from which endophytes are selected as inoculants for pasture (Powell and Daniel, 1978). As described above, this species does not produce the large resting spores typical of many VA fungi but can be recognised from other types in infections because it has very fine hyphae (0.5 μm thick, Hall, 1977a).

THE EFFECT ON PLANT GROWTH

The enhanced growth of plants infected with mycorrhizal fungi is often attributed to greater uptake of phosphorus by the infected plants (Hayman and Mosse, 1971), although the uptake of other nutrients may be involved, e.g. copper and zinc (Lambert et al., 1979); zinc (Gilmore, 1971); and sulphur (Gray and Gerdemann, 1973; Rhodes and Gerdemann, 1978a, 1978b; Cooper and Tinker, 1978). Safir et al. (1971) suggested that mycorrhizal plants were more resistant to drought than non-mycorrhizal plants but later attributed the effect to improved phosphorus nutrition (Safir et al., 1972). A possible relationship between the enhanced uptake of phosphorus by mycorrhizal plants and resistance to drought is that plants deficient of phosphorus are not as highly hydrated as plants with sufficient of the element

(Atkinson, 1973).

The relationship between the fungus and the host is a mutualistic symbiosis: the fungus provides the plant with nutrients and the plant provides the fungus with carbohydrates (Bevege et al., 1975; Cox et al., 1975).

Sanders and Tinker (1971, 1973) investigated the mechanism by which phosphorus uptake was increased in mycorrhizal plants. The mechanism was investigated by labelling the soil with ^{32}P and comparing the specific activity of phosphorus in mycorrhizal and non-mycorrhizal plants. It was concluded that both mycorrhizal and non-mycorrhizal plants absorbed from the same source of phosphate in soil solution. The work was confirmed using the same method by Hayman and Mosse (1972) and by Powell (1975a) who increased the number of endophytes tested. Barrow et al. (1977) incubated soil fertilized with phosphate at high temperatures to provide treatments where phosphate was firmly bound in the soil. The growth responses by mycorrhizal and non-mycorrhizal plants were compared when grown in soils which had been incubated for various lengths of time. The growth of mycorrhizal plants was improved (compared to the non-mycorrhizal plants) to the same extent when grown in soils that had been incubated for different periods and the results indicated that VA mycorrhizas tap the same source of phosphate as plant roots.

In further investigation of the mechanism by which VA mycorrhizas absorb phosphate, Sanders and Tinker (1971,

1973) measured the rate of uptake of phosphorus per unit length of root (inflow). The inflow of phosphorus in non-mycorrhizal onions was 3-4 mol $cm^{-1}sec^{-1} \times 10^{-14}$ and the inflow of phosphorus into mycorrhizal onions was 12-13 mol $cm^{-1}sec^{-1} \times 10^{-14}$. The maximum rate of inflow into non-mycorrhizal roots was calculated and was about equal to the measured values and the extra uptake of phosphorus by the mycorrhizal plants must have entered the roots via the external hyphae.

Roots often absorb phosphorus from the soil solution at a faster rate than the soil solution is replenished; a zone depleted of phosphorus extends around the root for about 1 mm (Tinker, 1975b). Mycorrhizal hyphae extend from the root and absorb phosphorus from the undepleted soil. Rhodes and Gerdemann (1975) found that mycorrhizal hyphae can translocate ^{32}P from distances of up to 7 cm from the root but Owusu-Bennoah and Wild (1979) measured depletion zones of 2 mm for mycorrhizal roots and 1 mm for non-mycorrhizal roots using autoradiography. The effect of the mycorrhiza was similar to a cylinder of root hairs of 1 mm long.

The mechanism by which VA mycorrhiza take up nutrients depends upon (a) the concentration of the nutrient in the soil solution being less than that required for maximum growth of the host; (b) a store of the nutrient which is in equilibrium with that in the soil solution and (c) a volume of soil in which the nutrient is not depleted , from which the external hyphae can tap (Tinker, 1975a).

The density of roots in grassland may be so great that the depletion zones of individual roots overlap so that plants do not benefit from the mycorrhizal association (Sparling and Tinker, 1975).

Recently Cress et al. (1979) studied the kinetics of phosphorus absorption by mycorrhizal and non-mycorrhizal tomato roots in solution culture. They found that mycorrhizal roots had a greater affinity for $H_2PO_4^-$ ions than non-mycorrhizal roots and that the greater absorptive area presented by mycorrhizal roots was not of major importance at phosphorus concentrations in solution similar to those expected in the soil. However, the study was undertaken in stirred nutrient solutions, but in soils where zones of phosphate depletion occur around roots, and where the greater absorptive area of mycorrhizal roots may be of more significance.

In some circumstances VA infections can reduce plant growth. Mosse (1973b) attributed depressions in growth of non-mycorrhizal plants when given high levels of soluble phosphorus to a build up of phosphorus in plant cells to toxic concentrations. Growth depressions in mycorrhizal plants occurred at lower levels of phosphorus than in non-mycorrhizal plants and this effect was explained because mycorrhizal plants took up more phosphate than non-mycorrhizal plants and the phosphorus in plant cells reached toxic concentrations with smaller amounts of added phosphate. With very high levels of phosphorus in the soil, growth depressions were

accompanied by uncontrolled infections, where attenuated hyphae ran irregularly through the root cortex. Similar effects have been recorded by Sanders (1975) when soluble phosphate was injected into the leaves of onions and by Crush (1976) where inoculation with mycorrhizal fungi caused growth depressions in legumes.

Cooper (1975) recorded transient depressions in growth of seedlings and suggested that they were caused by competition between mycorrhizal fungi and plant roots for phosphate in the soil.

Hall et al. (1977) found that growth depressions occurred when the concentration of phosphorus in the shoots of white clover was greater than 0.6% but this concentration was not toxic to non-mycorrhizal plants. Growth depressions occurred where levels of infection were less than 1%. The authors did not rule out the occurrence of toxic concentrations of phosphorus or competition between the plant and fungus in early growth. A simple explanation for growth depressions is that the fungus is a powerful sink for photosynthates from the host and receives material when the host does not benefit from nutrient uptake by the fungus. However, in a well developed infection where the fungus probably weighs less than 10% of the weight of the host root, it is unlikely that the fungus would take a significant amount of photosynthate from the host (Tinker, 1978) unless the metabolic rate of the fungus is very much greater than that of the host. More than one factor may cause growth

depressions but long lasting effects are unlikely to occur when the growth of the host is limited by phosphorus (Abbott and Robson, 1977b).

THE EFFECT OF NODULATION AND NITROGEN FIXATION

In 1944, Asai reported that nodulation of various legumes grown in steamed soil was dependent upon the addition of 5-10 g of unsterile soil and the subsequent formation of VA mycorrhizas but it was not until the 1970s that more detailed studies into the relationship between VA mycorrhizas and symbiotic nitrogen fixation were reported.

Soybeans grown in fumigated soils and infected with mycorrhizal fungi had a greater concentration of nitrogen in the leaves and protein in the seeds when compared with non-mycorrhizal plants (Ross and Harper, 1970; Ross, 1971). Schenck and Hinson (1973) treated nodulating and non-nodulating isolines of soybeans with mycorrhiza in fumigated soil and found that plant yield and the level of protein in the seeds was increased only in the nodulated isoline. Speculation that VA mycorrhizas may fix nitrogen was abated, at least for *Glomus macrocarpus*, the endophyte used in the study.

The number of nodules and the activity of nitrogenase (measured by acetylene reduction) is enhanced when legumes are inoculated with both rhizobia and VA endophytes and grown in sterile media, which is low in available phosphate, when compared with plants inoculated with only rhizobia

(Crush, 1974; Daft and El Giahmi, 1974, 1975; Mosse et al., 1976). In unsterile soils the degree that nodulation was stimulated by introduced fungi was inversely correlated with the extent of mycorrhizal infection by indigenous fungi (Mosse et al., 1976). In other words, indigenous fungi can also increase the number of nodules. The effects were thought to result from the better phosphorus nutrition of the host (Crush, 1974; Daft and El Giahmi, 1974, 1975; Abbott and Robson, 1977b).

However, there may be an additional stimulatory effect to nodulation and nitrogen fixation by mycorrhizas. Mosse (1977a) found that in one soil there were more nodules on the roots of Stylosanthes inoculated with introduced fungi than there were on plants infected with indigenous endophytes although the phosphorus concentrations in the plants were about the same. With another soil there were few nodules on plants well infected with introduced endophytes which had a level of phosphorus in the tissues considered adequate for nodulation. These results could indicate that endophytes affect nodulation other than by improving phosphorus uptake or they could be explained if the endophytes differed in the time taken to infect and spread throughout the root system. Early infection and phosphorus uptake would lengthen the time for nodulation and increase numbers compared with nodulation on plant roots inoculated with an endophyte which infects slowly. The level of infection in plant roots caused by endophytes in an experiment that is harvested

once may not differ even if there have been differences in the rates of infection. Smith and Daft (1977) measured the growth of nodulated Medicago sativa with and without mycorrhiza. They found that mycorrhizal plants were better nodulated and had higher rates of nitrogenase activity than non-mycorrhizal plants two weeks after nodulation, but the phosphorus concentration in the plants and growth of the mycorrhizal and non-mycorrhizal plants differed only after 7 weeks and 10 weeks respectively. In a later study, with subterranean clover, nodulation and nitrogen fixation were similarly enhanced before growth responses were apparent (Smith et al., 1979). The extra effect of mycorrhiza on nodulation and nitrogen fixation above that of better phosphorus nutrition may be caused by uptake of other elements necessary for nitrogen fixation, e.g. copper and manganese (Ross and Harper, 1970) or early mycorrhizal infection may increase the supply of photosynthate to the nodules (Smith et al., 1979). Bagyaraj and Menge (1978) suggested that there may be associations between the free living nitrogen fixing organism Azotobacter chroococcum and the external mycelium of mycorrhizas. This could explain the reports (Smith and Daft, 1977; Smith et al., 1979) that nitrogenase activity was stimulated in mycorrhizal plants before effects on plant nutrition were evident.

For early reliable establishment of white clover in hill pastures, seedlings must nodulate quickly so that fixed nitrogen can supply the plant as soon as possible

after the reserves of nitrogen in the seed are depleted. Infection threads were formed 5-6 days after white clover was inoculated with rhizobia and the first nodules appeared after 8-16 days (Vernon, 1978). Uptake of phosphorus by mycorrhizas has been detected 21-23 days after inoculation (Gerdemann, 1968; Mosse et al., 1969; Sanders and Tinker, 1973; Owusu-Bennoah and Wild, 1979). This would be too late to have the most beneficial impact on nodulation during the establishment of clover if phosphorus uptake is the only stimulatory effect of mycorrhizas. A few moderately sized nodules may be sufficient to support an actively growing clover plant and a greater number of nodules on mycorrhizal plants may not be beneficial (Newbould and Haystead, 1978). Mosse (1977b) found that approximately 40 small nodules, which did not fix nitrogen, formed on the roots of Stylosanthes in an unsterile soil when infected by indigenous fungi (15-20% root infection), but fewer large nodules formed on plants inoculated with mycorrhizal fungi (80% root infection) and the nodules fixed nitrogen. It was suggested that indigenous, inefficient rhizobia were able to infect and nodulate plants with a lower concentration of phosphate in the tissues than introduced strains of Rhizobium which were efficient at fixing nitrogen. However, in the experiment all of the Stylosanthes plants were inoculated with Rhizobium and it may be that the large and small nodules had been formed by the introduced strain of Rhizobium.

An interesting possibility from mycorrhizal infection of pasture legumes is that nitrogen from the legume may pass from the clover root through the fungal hyphae to the companion grass. Infections are thought to spread from root to root in the soil regardless of species (Tinker, 1975a) and there is generally a high concentration of nitrogen in the legume and a lower one in the grass. There is a little evidence that transfer may take place between species; Woods and Brock (1964) injected ^{32}P into stumps of Red Maple and found the radioisotope in 19 other species up to 8 m away and Hirrel and Gerdemann (1979) found that ^{14}carbon was transferred between mycorrhizal onion plants although the transferred carbon remained in the roots of the recipient plant and it was uncertain whether the element was released to the plant.

INTERACTIONS WITH MICRO-ORGANISMS OTHER THAN RHIZOBIUM

Studies concerning the relationships between VA mycorrhiza and micro-organisms have followed two main lines of investigation, the interaction with plant pathogens and the interaction with organisms in the rhizosphere.

Endomycorrhizal plants are known to be more susceptible to virus diseases than non-mycorrhizal plants, probably because the virus is better able to multiply in plants that are well nourished (Schönbeck and Schnizer, 1972; Daft and Okusanya, 1973). Some fungal diseases in

plants are associated with plants with an imbalanced nutritional status. Mycorrhizal plants may well be more resistant to such diseases than non-mycorrhizal plants (Walker, 1969; Strobel-Mathre, 1970), and may explain why the spread of Fusarium oxysporum in the shoots of tomato was restricted more in mycorrhizal than non-mycorrhizal tomato (Dehne and Schönbeck, 1979). Diseases caused by root pathogens can be increased in mycorrhizal plants because the mycorrhizal infection can damage the root and allow a pathogen to gain entry, e.g. Phytophora root rot was probably increased in mycorrhizal soybean because vesicles produced by the fungus split the root (Ross, 1972). Competition between VA fungi and pathogens, like nematodes, for root space can occur (Fox and Spasoff, 1972; Baltruschat et al., 1973; Schenck and Kinloch, 1974) and changes in the composition of the chemicals in the rhizosphere can influence the development of pathogenic fungi (Baltruschat et al., 1973).

The relationships between rhizosphere micro-organisms are complex and few studies have been undertaken. Hormones produced by bacteria are known to influence levels of infection (Azcon et al., 1978a; Bagyaraj and Menge, 1978) and it has been suggested that mycorrhizal infection may alter the composition of root exudates and affect the rhizosphere population (Barea et al., 1975). There are interactions between VA fungi, rhizosphere, micro-organisms and plant species (Christie et al., 1978). The relative abundance of micro-organisms associated with

a plant species grown alone is altered in the presence of another plant species, although VA mycorrhizal infection of a species was less affected by neighbouring plant species than populations of bacteria and root surface fungi.

THE DEVELOPMENT OF INFECTION

Sutton (1973) described the development of VA mycorrhizal infection in a wide range of crop plants in a controlled environment and in the field. He described a three-phase pattern of development, a lag phase, an extension phase and a constancy phase, which was common to a diversity of host plants. The lag phase corresponds to the time when seedlings were making extensive root growth while fungal spores germinated and infections were in the early stages of development. The extension phase coincided with rapid increases of dry weight in the host when the fungus formed an extensive mycelium in the soil and reinfection by the hyphae caused the fungus to spread. The constancy phase occurred when the host began to fruit but at this stage the percentage of root that was infected did not change. Also during this latter phase the fungus often sporulates (Furlan and Fortin, 1977). The extension phase in biennial hosts was longer than in annual hosts, but there is little information for perennial crops. A mathematical model of the spread of infection resulted in a sigmoid curve (Tinker, 1975a), similar to those obtained experimentally

by Sutton (1973).

The shape of the infection curve may be affected by environmental variables, particularly factors which affect root growth. Root growth of clover is influenced by soil moisture and temperature, flowering and grazing (Masterson and Murphy, 1976; Caradus and Evans, 1977). Sparling and Tinker (1978a) found that about half the root cortex of grasses in upland pasture was infected and that, even though there was a considerable amount of root turnover, seasonal changes caused small differences in the level of infection. Similar levels of infection may occur in white clover grown on the hill. The sites of Sparling and Tinker (1978a) were fenced to prevent grazing as frequent defoliation could influence the levels of infection (Daft and El Giahmi, 1978).

MEASUREMENT OF INFECTION

For a greater understanding of the effects of mycorrhizas on plant growth, the ways of measuring infection and the time of sampling must be considered. The percentage of infected root is the measurement that is most often related to plant growth and nutrient uptake but Daft and Nicolson (1972) and Mosse (1973b) and others have not found that it correlates well with growth. Daft and Nicolson (1972) measured percentage root infection, spore production and root pigmentation and found that the method of assessment which correlated best with plant growth was the number of chlamydospores produced by the

external mycelium but this method was confined to endophytes that form conspicuous ectocarpic spores and where plants are grown in rooting media that is free of spores and from which the spores can be easily extracted (Tinker, 1975a). Assessment of the yellow pigmentation of the root by eye was easy and quick and gave a fairly good correlation with plant growth but the method was restricted because not all host roots turn yellow when mycorrhizal, and field samples are rarely yellow. Measurement of root infection was found to be better correlated with plant growth when related to the size of the root system than when expressed as a percentage of root infected.

Abbott and Robson (1977b) also found that the weight of mycorrhizal root related better to plant growth than percentage of infected root. Measurements that are cumulative, i.e. spore number, weight/length of infected root, are more likely to correlate with plant root than the percentage of infected root which is a spot measurement. Sanders and Tinker (1973) calculated a length of about 80 cm external hyphae per cm infected root and found that the amount of hyphae per centimetre of root was uniform for a range of infected root lengths for a particular fungus. If the calculations are based upon the correct assumptions, the length of infected root should correlate well with plant growth.

Hall (1977a) noted that, when there were few arbuscules formed in infections of Glomus pallidus, Coprosma robusta did not gain any benefit from the

association and he suggested that arbuscule formation in plant roots may be related to mycorrhizal efficiency. In a later report, Hall (1977b) states that infections in white clover were predominantly vesicular at high levels of soil phosphate where plants did not benefit from inoculation but were predominantly arbuscular when infection enhanced growth.

Arbuscule counts require detailed examination of roots under the compound microscope and may not be justified in all experiments. Measurement of the length of infected root is more difficult than measurement of the weight of infected root but the latter value may correlate as well with the weight of the plant. It is often impossible to retrieve the whole of a plant's root system from the field, particularly in stoloniferous plants like clover, so infection cannot easily be related to the size of the root system and, therefore, the most practical method of assessment of infection from field samples is the percentage of infected roots.

The previous section described the development of the infection. It is obvious that for a fuller analysis of growth responses caused by different endophytes under varying environmental conditions, serial harvests must be made to follow differences in the rate of infection. Sanders et al. (1977) found that three endophytes produced approximately the same amount of external mycelium per centimetre of infected root and the inflow of phosphorus into the root was similar. Small differences

between the endophytes were accounted for by differences in the rates of spread of the infection. A fourth endophyte which did not promote growth in the host spread slowly and probably also absorbed and transferred phosphate inefficiently.

FACTORS WHICH AFFECT THE SYMBIOSIS

VA fungi grow both in the soil and in the plant and are affected by conditions in both environments. A complication arises when trying to understand how the fungus is affected by an environmental variable; there may be either a direct effect on the external hyphae or an indirect effect mediated via the plant (Tinker, 1975a), e.g. pH of the soil. Does a low soil pH reduce infection by direct damage to the external mycelium or indirectly by increasing the solubility of phosphates or by changing the growth and metabolism of the host? The main factors which affect the symbiosis are the host plant, the nutrient level in the soil (including soil pH and moisture level), temperature, light intensity and photo-period, and the form and density of the inoculum and pesticides. There are numerous reports of the effect of application of soluble phosphorus to the soil and a few reports concerning other environmental variables. In most cases it is not possible to relate the effect of the variable to infection, phosphorus uptake and transfer or spore production by the fungi.

The host plant

The benefit derived from VA infection by the host plant depends on the growth rate of the plant, the morphology of the root and development of root hairs and the percentage of the nutrient required by the host tissue for active growth. Plants which grow slowly require less phosphorus per unit time than plants that grow more quickly if the optimum concentration of phosphorus in the plant tissue is about the same and if the root morphology of the plants is similar. The phosphorus in a soil may be replenished with sufficient speed to satisfy the need of a slow growing species and such a plant would not benefit from mycorrhizas; the greater the growth rate of a plant, the greater the benefit from mycorrhizas. Differences in response to phosphate by Urtica dioica and Deschampsia flexuosa may be accounted for by differences in growth rate (Nassery, 1970).

Baylis (1970, 1972b) suggested the plants which benefit most from VA mycorrhiza have either poorly developed or no root hairs whereas plants with long root hairs and finely divided roots gain little benefit from mycorrhizas. The work of Crush (1974) with legumes supports the theory. Grasses have finely divided roots with long root hairs and do not respond to inoculation (Baylis, 1972a; Sparling and Tinker, 1978b). Clover has a more coarsely branched root system with shorter root hairs than ryegrass (Evans, 1977) and greatly

benefits from mycorrhiza inoculation (Crush, 1974; Powell, 1976a; Sparling and Tinker, 1978c). The yield of clover when grown alone or with ryegrass was greater when mycorrhizal than when non-mycorrhizal but growth of ryegrass when mycorrhizal was the same or less than when non-mycorrhizal (Crush, 1974; Hall, 1978a). In the experiments of Hall (1978a), and possibly in one of the experiments of Crush (1974), ryegrass was deficient in nitrogen, and was therefore unable to respond to mycorrhizas.

Baylis (1972b) stated that plants most likely to retain root hairs are those that undergo periodic and rapid root extension, e.g. after dessication or grazing, when flushes of root growth may outstrip the spread of the mycorrhiza. Tinker (1975b) discusses the relative merits of root hairs versus VA mycorrhiza. Root hairs have short lives and they may be less effective than hyphae because individual depletion zones may overlap. Hyphae are more dispersed and therefore it is less likely that they would compete for phosphorus. Although the work of Owusu-Bennoah and Wild (1979) suggests that hyphae do compete with each other for uptake of phosphorus in a similar manner to root hairs.

Plants that grow with a relatively low concentration of phosphorus in the tissues will be less dependent on mycorrhizas for growth than plants that require a greater concentration of phosphorus in the cells for active growth, all other things being equal. Hall (1978b) investigated the effects of mycorrhizas on two cultivars of Zea mays

and a cultivar of Zea mays x robusta. The growth of one cultivar of Zea mays and the Zea hybrid was enhanced when mycorrhizal in an unfertilized soil. The other cultivar of Zea mays outgrew the other two types and did not respond to mycorrhizal inoculation even though the roots of the three cultivars were infected at similar levels. The ability of the cultivar to grow well in soil low in phosphorus when non-mycorrhizal was attributed to the lower concentration of phosphorus required by the shoot for growth and better root growth compared with the other two cultivars.

Conditions in the soil

The level of phosphorus and nitrogen in the soil and the pH and wetness of the soil have been reported to affect mycorrhizas. Alteration of the levels of the aforesaid factors by application of lime and fertilizers to the soil can therefore affect the symbiosis.

(a) Phosphorus. The enhanced growth which generally occurs when plants are inoculated with VA fungi is reduced by application of soluble phosphate to soils (Daft and Nicolson, 1966; Baylis, 1967; Murdoch, Jackobs and Gerdemann, 1967) and when plants are inoculated in soils with high levels of 'available' phosphorus (Mosse, 1972). Daft and Nicolson (1969a) found that soluble phosphate reduced the percentage of root infected by the fungus and the number of spores produced.

The effect of soil phosphorus is a good example of how an apparently direct effect of phosphorus in the soil

may be an indirect effect of the concentration of phosphorus in the plant. To clarify this, Sanders (1975) injected soluble phosphate onto the leaves of mycorrhizal onions. The resultant increase in the phosphorus status of the plant reduced the rate of spread of the fungus and the intensity of mycorrhizal infection, reduced the density of the external mycelium and depressed the translocation of phosphorus to the host plant, and so confirmed that the phosphorus status of the plant affected the symbiosis.

Menge et al. (1978a) came to the same conclusion when high phosphorus fertilization of one half of the root system of Sudan grass (using the split-root technique) increased the phosphorus status of the whole plant and reduced the number of spores produced by the fungus growing on the other half of the root system in soil without added phosphorus. In another experiment where a portion of the roots of plants growing in variously P-fertilized soil was allowed to grow into soil containing a different amount of phosphorus, the numbers of vesicles, arbuscules, spores and the length of hyphae per cm root in the treated portion of root were inversely correlated with the phosphate level in the plant and not with the concentration of soluble phosphorus in the rooting medium.

Jasper et al. (1979) stressed the importance of the time that measurements of phosphorus are made and the tissues that are analysed. They found that the level of

infection was correlated with the phosphorus concentration in the roots at the time of infection and not with the concentration of phosphorus in the plants at harvest. However, there is some contradictory evidence which suggests that both the concentration of phosphorus in the shoot and the level of soluble phosphorus in the soil affect infection. Azcon et al. (1978b) transplanted non-mycorrhizal lettuce seedlings with a range of phosphorus status, into soil containing different levels of soluble phosphate and inoculated the plants with VA fungi; infection was reduced both by high levels of soil and plant phosphorus.

In short term experiments Ratnayake et al. (1978) found that root exudation of soluble amino acids and reducing sugars from Sorghum vulgare and Citrus aurantium decreased as applications of soluble phosphate increased and suggested a mechanism by which applied phosphorus could reduce the level of mycorrhizal infection. They suggested that the permeability of cell membranes increases when plants are deficient in phosphorus, and hypothesised that exudates sustained early growth and infection of VA fungi at low levels of phosphorus but were insufficient to support infection when greater levels of phosphorus were applied.

Therefore, the overwhelming amount of evidence suggests that the phosphorus level in the plant influences the degree of infection much more than the level of soluble phosphorus in the soil.

The form of phosphorus applied can greatly influence the response by plants to mycorrhizal inoculation. Growth of plants was stimulated when inoculated with mycorrhizal fungi when slowly available forms of phosphorus were applied to soils at rates where the equivalent level of soluble phosphorus inhibited fungal development (Daft and Nicolson, 1966; Murdoch, Jackobs and Gerdemann, 1967; and others). It seems that the amount of soluble phosphorus in the soil which is available to the plant is the factor which determines the effect of mycorrhizas on plant growth and not the total amount of phosphorus. Parunan et al. (1980) found that, when soluble and insoluble forms of phosphorus were applied at levels which gave the same concentration of phosphorus in the shoot, mycorrhizas had similar effects on growth. Mycorrhizas were not able to increase growth when phosphorus was not available for uptake or when phosphorus supply did not limit growth.

(b) Nitrogen. Hayman (1970, 1975) reported that applications of nitrogen fertilizer reduced the spore number in soils and the level of infection in arable crops. Redhead (1975) found that nitrogen fertilizer reduced the number of vesicles and the level of infection but increased spore production. Sparling and Tinker (1975) did not detect a change in infection levels when 125 kg/ha Nitram (approximately 26% N) was applied to an acid grassland. Nitrogen fertilizer may enhance the beneficial effect of mycorrhizas by increasing growth and reducing the

concentration of phosphorus in the plant.

(c) The pH of the soil. The pH of the soil variously affects the infection and spore production by the VA fungi (Peuss, 1958; Mosse, 1972; Krucklemann, 1975; Sparling and Tinker, 1975). It seems probable that different fungi have different pH optima (Mosse, 1972). The effect of pH may be partially caused by the alteration in the solubility of phosphorus compounds at different hydrogen ion concentrations, particularly in fertilized soils (Mosse et al., 1976; Graw, 1979).

(d) The wetness of the soil. It is a common observation that mycorrhizas are absent from plant roots growing in waterlogged soils (Asai, 1934; Stahl, 1949; Maeda, 1954), although mycorrhizal plants have been noted in swamps and bogs (Rayner, 1927; Dowding, 1959). Khan (1974) reported mycorrhizas on Ipomoea cornea in Pakistan during the dry season but during the monsoon, when soils were waterlogged, the plants were non-mycorrhizal. Redhead (1975) noted that root infection of Khaya grandifolia was not greatly affected by moisture regime but root dry weight was restricted in very dry soil and waterlogged soil. The amount of infected root was, therefore, less in very dry and waterlogged soils and spore production was similarly affected. The author suggests that the external mycelium may be affected more by fluctuations of water level in the soil than the hyphae in the root.

Temperature

Temperature can greatly modify the effects of VA mycorrhiza on plant growth. The rate of infection by Glomus calospora was increased as temperature increased with a range of night/day temperatures from 11/16^{0}C to 21/26^{0}C (Furlan and Fortin, 1973). Mycorrhizas increased the growth of onions at temperatures favourable for plant growth but the growth stimulation was reduced when the temperature was lowered (Hayman, 1974). Schenck et al. (1975) noted that spores from three species of VA fungi had a different optimum temperature for germination. Schenck and Schroder (1974) observed that Gigaspora gigantea was common in summer crops in Florida when soil temperatures were around 31^{0}C. Optimum temperature for development of infection and spore production was investigated over a range of soil temperatures and occurred between 30-35^{0}C.

Smith and Bowen (1979) found that root temperature affected the number of fungal entry points on roots of subterranean clover and temperature is thought to affect the rate at which phosphorus is translocated in the hyphae (see Tinker, 1978).

Light intensity and photoperiod

The growth of onions was stimulated more by mycorrhizas formed by Glomus fasciculatus at approximately 100 Wm^{-1} than at 52 Wm^{-1} and at low light intensity there was more growth with an 18 h day than a 6 h day. The

effect was not solely an effect of light intensity and duration on plant growth because mycorrhizal onions were affected more by low light intensity than plants given soluble phosphate (Hayman, 1974). The results obtained by Furlan and Fortin (1977) for onions infected with Gigaspora calospora do not agree with Hayman (1974) when a different endophyte was used but do emphasise the importance of light intensity on the symbiosis. Infection was more rapid at low light intensities (40 W/m^2 or less) than at higher light intensities and the production of spores increased with light intensity. The greatest stimulation of plant growth occurred at 40 W/m^2 LI (Furlan and Fortin, 1977).

Daft and El Giahmi (1978) measured the effects of light intensity and duration on tomato and maize, of periodic defoliation on grasses and the effect of light intensity on maize. The authors concluded that treatments which affect the supply of carbohydrates to the fungus affect the development of the mycorrhiza.

Type and density of inoculum

Daft and Nicolson (1969b) demonstrated the effect of inoculum density on plant growth. They inoculated tomato with 3 to 225 spores per plant. All inoculations stimulated growth but the more concentrated inoculum stimulated more growth. The final level of infection was not influenced by the concentration of spores.

Hall (1976) inoculated seedlings of Coprosma robusta with either 50 spores or 2 ml of roots infected with either

Acaulospora laevis or a species of Glomus. (The infective material had approximately the same amount of fungal material.) Growth was stimulated earlier with Glomus than with Acaulospora and chopped roots were more effective than spores. Glomus infected plants were larger than those infected with Acaulospora even though the latter infection seemed as effective once established. Johnson (1977) found hyphae infected Coprosma robusta earlier than fresh mycorrhizal roots; fresh roots infected earlier than old roots (3 months) and old mycorrhizal roots infected at about the same time as spores.

In the literature where differences between endophytes in increasing plant growth are reported, sufficient information about the age, amount and proportion of various inoculum types in the inoculum is rarely given. The work above emphasises the importance of standardising the amount of inocula applied before an endophyte can truly be said to be more efficient than another. Similarly, inoculation of plants in unsterile soil either by transplanting preinoculated plants or by placing high density inoculum near to the seed may increase plant growth by early infection rather than any genetic difference between introduced and indigenous endophytes.

Pesticides and herbicides

Herbicides are applied to hill land for two reasons: to destroy indigenous vegetation before soil is cultivated

and reseeded and to eradicate bracken (Pteridium aquilinum) (Newbould, 1974; Davies et al., 1979). Other pesticides are rarely used. The use of pesticides for intensively grown crops is more usual and much of the work has concentrated on effects of fungicides on VA mycorrhizas for use in agriculture and as an experimental tool to obtain soils free from mycorrhizas with a minimal effect on other micro-organisms (see Nesheim and Linn, 1969; Jalali and Domsch, 1975; Kleinschmidt and Gerdemann, 1972; Smith, 1978 and Menge et al., 1978b,1979).

To the author's knowledge only one paper deals with the effect of herbicides. Burpee and Cole (1978) applied the herbicides Alachlor and Influral into soil as a spray at the recommended rate (2 kg/ha) and at double the recommended rate. The lower application had no effect on VA fungal development or on the growth of soybean roots. Root growth was inhibited at the higher rate but mycorrhizal fungi were unaffected.

VARIATION IN ENDOPHYTES

Floate (1977) divided the limitations to improved pasture production on hill land into two categories: those that are permanent and those that can be altered. Climatic limitations, temperature, light intensity and rainfall (which leads to waterlogged soils) are permanent and these factors are known to affect the mycorrhizal symbiosis (see previous section). The effect of light intensity on the symbiosis is probably almost entirely

mediated through photosynthesis by the plant but it may be possible to select endophytes which are tolerant of low temperatures or wet soils and are also efficient in the uptake of phosphorus. As discussed in the previous section, information published by different workers suggests that endophytes differ in their tolerances of environmental factors. However, some of the variations may have been the result of the different experimental techniques used. In this section the results of experiments are discussed where the effects of environmental variables were compared under standard conditions.

Endophytes which vary in their tolerance to temperature must occur because endomycorrhizal plants are found in vegetation from tropical rain forest and tundra (Hayman, 1978) and Schenck et al. (1975) found that the optimum temperature for the germination of spores varied and was related to the region from which they were collected.

Acidity and low fertility of the soil are temporary limitations to hill land production, as is the low production and quality of indigenous species (Floate, 1977). When lime and fertilizers are applied to hill soils and white clover and better quality grasses are sown, the indigenous endophytes may not be the most efficient strains at phosphorus uptake in the altered conditions. Mosse (1972, 1977b) has demonstrated differences between endophytes in their ability to take up phosphorus from soils and in the relative efficiencies of strains of fungi in

their ability to take up phosphorus in different soils. Powell (1977a) observed similar differences between endophytes when clover was inoculated with six strains of fungi in four hill soils. Skipper and Smith (1979) found that the relationship between cultivars of soybean and species of mycorrhizal fungi was affected by the pH of the soil.

There is some work investigating the effects of applications of fertilizers to pasture on the effectivity of the population of endophytes in the soil. Sparling and Tinker (1978c) found that two endophytes indigenous to acid grassland differed in tolerance of pH and phosphate level in pot experiments. The fine endophyte was dominant on limed soil and the coarse endophyte was dominant on soil which received a dressing of basic slag. The indigenous endophytes seemed able to improve the uptake of phosphorus by clover over a range of applications of soluble and rock phosphate, although the effects were not consistent. Rangeley and Holding (unpublished) found in pot experiments with a peaty podzol that the proportion of the infection of clover roots by the fine endophyte was less when superphosphate was applied to the soil. There was also a marked reduction in the proportion of infection caused by coarse endophytes when the level of water applied to the soil was increased from 40 to 80% field capacity. Therefore endophytes found in the same environment may have different tolerances to environmental variables.

Crush (1978) grew Huia and a hill country type of white clover in soil from scrub, an undeveloped pasture and a developed pasture (the soils differing most in the amount of available phosphorus). When the clover was infected with indigenous mycorrhiza, the plants of each clover/mycorrhizal combination were transplanted into each of the soils, which had previously been sterilized. In general, the endophyte from the well developed pasture promoted more growth than the other mycorrhizal types on Huia and less growth on the hill country type clover when compared with the other endophytes. The endophyte from the scrub soil promoted growth more than the one from the developed site on the hill country clover and therefore there was a strong endophyte x clover type interaction. As the fertility of the soil increased, it could have been expected that each endophyte would be the most efficient in promoting plant growth in the native soil but this was not the case. The overriding influence was the host x endophyte interaction.

Porter et al. (1978) studied the infectivity and infectiveness of spores of indigenous endophytes from soil that had received none or 224 kg superphosphate/ha/year for 10 years. Prior to that date both treatments had received 150 kg superphosphate/ha/year for 15 years. There was no difference in infectivity or effectiveness in subterranean clover of the two VA fungal populations when grown in soil given a range of levels of phosphorus fertilizer. However, the endophytes were isolated from

pasture with a history of application of superphosphate. Endophytes isolated from virgin forest or scrub may not be tolerant of the increased fertility of a developed pasture.

The limited data presented here suggests that indigenous fungi may be as efficient at phosphorus uptake in fertilized soils as in unfertilized soils, that host endophyte interactions may be as important as soil endophyte interactions. Mycorrhizal fungi are coencytic and probably heterokaryotic. Little is known about the genetics of such fungi because they are very difficult to work with. Mycorrhizal fungi are particularly difficult because they cannot be grown alone on artificial media. It is possible that when conditions change nuclei with the most adaptive genotype become dominant. The genetics of a mycorrhizal fungus may be the genetics of a population which is stable in a wider range of conditions than an individual with a single genotype. A logical step from selecting endophytes with desirable qualities is to breed strains which have a number of the qualities desired. The genetic system of the fungi would make this almost impossible as the fungi do not anastomose and are not known to produce sexual reproductive spores.

THE PRACTICAL APPLICATION

The exploitation of vesicular arbuscular mycorrhizas in agriculture, particularly the growth of white clover on hill land, offers the possibilities of more reliable

establishment and early development of nitrogen fixation and a greater yield of produce for the same input of phosphorus fertilizer or the same yield with less fertilizer. Plants infected with VA mycorrhizas would then be more efficient in fertilizer uptake and dry matter production (Abbott and Robson, 1977b; Mosse, 1977b; Powell and Daniel, 1978). Whether or not the association would be economically efficient depends upon the cost of establishing the symbiosis and the level of fertilization at which growth stimulation occurs. Inoculation of crops in the field with mycorrhizal fungi can be of benefit if the density of indigenous inoculum in the soil is low as is probably the case during the establishment of clover in reseed hill pastures (see Occurrence), or if an improved strain is selected (Tinker, 1975a).

Some field data suggests that inoculation in soils with a low density of inoculum can increase levels of infection and growth. Khan (1972, 1975) transplanted mycorrhizal and non-mycorrhizal wheat and maize seedlings into the field on unfertilized land which had grown Chenopodiacous weeds. At harvest, the mycorrhizal plants were heavier than the non-mycorrhizal plants. Black and Tinker (1977) grew potatoes on land that had been left fallow for two years so that the density of spores was low in the soil (1 spore/ml soil). The potatoes were inoculated with soil from a nearby plot in which barley had been grown where the density of spores in that soil

was greater (13 spores/ml soil) and large increases in yield resulted from inoculation in the absence of phosphorus fertilizer.

When inoculum was placed directly below the seed of onions, lucerne and barley, at a site that had been fallowed for 6 years and contained few mycorrhizal spores, the three crops yielded more when inoculated. Lucerne and onion benefited more than barley, which was to be expected from the root morphology of the plants (Owusu-Bennoah and Mosse, 1979).

Powell (1977b) has successfully inoculated white clover with Glomus fasciculatus E3 in the field by sowing seed above pads of soil containing inoculum. E3 caused more growth of white clover than indigenous fungi at a site with a southerly aspect but caused less growth of plants at a site on a northerly aspect. However, transplanted seedlings infected with E3 at the site with the northerly aspect tended to increase growth when compared with transplants infected with fungi indigenous to the soil. The author suggested that the disparity in results was due to slow infection by E3 in the field, but not in the transplants. Hayman and Mosse (1979) have found responses to mycorrhizal infection in upland Wales when mycorrhizal and non-mycorrhizal white clover plants were transplanted.

The use of mycorrhizas in agriculture is restricted by the scale of the operation as production of inoculum in large quantities is difficult and methods of inoculation

of crops have not been fully developed. The production of inoculum is restricted because the fungus is an obligate symbiont, and identification and maintenance of disease free cultures is time consuming and requires a great deal of space. There have been successful inoculations of seedlings in citrus nurseries where fumigation of soil to kill pathogens also kills mycorrhizal fungi. Infected seedlings were planted at intervals amongst non-mycorrhizal seedlings and the infection spread from root to root in the nursery bed (Kleinschmidt and Gerdemann, 1972). Owusu-Bennoah and Mosse (1979) found that an endophyte spread 22 cm from the point of inoculation in an arable soil in one season. Daft and Hacskaylo (1977) discuss the possibility of planting mycorrhizal broad leaved trees for revegetating areas of poor fertility. Black and Tinker (1977) showed it was possible to place inoculum in potato trenches and increase yield and this should be possible for other drilled crops.

White clover seed is broadcast when hill pastures are reseeded and where soils are too wet or stony to cultivate the seed is left on the soil surface. An ideal method of inoculation would be to pellet the seed with mycorrhizal fungi at the same time as the seed is inoculated with Rhizobium. Application of seed and the two inoculants would be a convenient and economical method of application, and close contact between seed and the mycorrhizal propagule may allow sparing use of the inoculum. Mycorrhizal propagules are relatively large and pelleted

clover seed produced up till now is heavy and bulky and would be difficult to handle with existing machinery. Hall (1979) has demonstrated that pellets of soil that weighed 1.4 g and contained infected roots, pieces of hyphae and 20 spores/g soil were infective and stimulated plant growth up to 28 days after they were prepared, although newly prepared pellets most effectively stimulated growth. It was suggested that root segments and hyphae had died in 14-28 day old pellets and only spores survived. Powell (1979) demonstrated growth responses from clover seed pelleted with mycorrhizal fungi in the field when hand sown on small areas.

LINES OF INVESTIGATION IN THE EXPERIMENTS

The experimental work described here studied the effects of inoculation of white clover with several types of mycorrhizal fungi in three hill soils in the laboratory and the field. In the laboratory experiments, particular emphasis was paid to the effects of mycorrhiza on the phosphorus and potassium nutrition of the plant and on nodulation and nitrogen fixation. In both field and laboratory experiments, where possible, the success or otherwise of colonisation by introduced endophytes was investigated.

The following section describes the endophytes and soils used for the experiments and the methods employed where they differ from those described in Part I.

MATERIALS AND METHODS

(where different from those described in Part I)

LABORATORY EXPERIMENTS

Treatments

Mycorrhizal and fertilizer treatments given in the pot experiments are summarised in Table 33 with the cultivar of white clover that was grown.

The fertilizer treatments are described in detail for individual pot experiments in Appendix 12.

Soils

In addition to two of the soils used for the nutrient experiments, the peat from Lephinmore and the brown earth from Sourhope, a second brown earth (Giffnock series) from the Cleish Hills, Fife, was used. Some characteristics of the three soils are given in Table 34.

Plants

Two cultivars of white clover 'Grasslands Huia' and 'Aberystwyth S184' were grown (Tables 33 and 34). Seeds were either sown directly into 10 cm diameter pots containing 150 g of damp peat or 300 g of damp brown earth, or sown in sand containing mycorrhizal inoculum and transplanted singly into 7.5 cm diameter plant pots containing 120 g peat or 200 g brown earth soil.

Table 33. A summary of the fertilizers, inoculants and cultivars of white clover used in the mycorrhizal laboratory experiments (more details of the fertilizers applied are given in Appendix 12)

Experiment	Fertilizers applied (kg/ha) N (as NH_4NO_3)	P Level	P Chemical	K (as KCl)	Mg (as $MgSO_4.7H_2O$)	Lime (as $CaCO_3$)	Inoculant	Cultivar
8	None	40 160	$Ca_3(PO_4)_2$	160	None	2900	Coarse endophyte baited from the peat	Huia
9	None or 200 ppm	0 40 160	$Ca_3(PO_4)_2$	0 40 160	10	2715	*G. mosseae* L1	Huia
10	¼ strength Dart and Pate nutrient solution - plus nitrogen : minus phosphorus (Dart and Pate, 1959)							Huia
11	None	13	Gafsa phosphate	59	None	2715	*G. mosseae* L1 *G. fasciculatus* E3	Huia S184
12	None	13	Gafsa phosphate	59	None	678	*G. mosseae* L1	Huia
13	None	50	Ground superphosphate	100	None	4000	*G. mosseae* L1 *G. mosseae* *G. macrocarpus* *G. clarus*	S184

Table 34. Some characteristics of the soils used in the mycorrhizal experiments

Soil	pH	Loss on ignition (%)	Bulk Density (g/cm^3) 0-10 cm depth	Ammonium acetate extractable nutrients $\mu g\ cm^{-3}/mg\ 100\ g^{-1}$						
				K	Ca	Mg	P	Al	Fe	Mn
Lephinmore peat	3.8	86	0.10	15/15	69/69	37/37	1/1	15/15	12/12	7/7
Sourhope brown earth	4.3	25	0.65	486/75	307/47	167/26	39/6	116/18	14/2	43/7
Cleish brown earth	4.8	18	0.61	107/18	982/161	59/10	2/0.1	273/45	56/9	29/5

N.B. Extractable nutrients are quoted in terms of both weight and volume of the soils

Mycorrhizal fungi

The mycorrhizal inoculants were:

I A mixture of endophytes baited from Lephinmore peat with maize. Most of the endophytes had coarse hyphae.

II *Glomus fasciculatus* E3 (Gilmore, 1968; maintained on onions). Kindly supplied by Dr. B. Mosse, Rothamsted Experimental Station.

III *Glomus mosseae* L1 (El Giahmi *et al.*, 1976; maintained on maize). Kindly supplied by Dr. M.J. Daft, Dundee University.

IV *Glomus caledonius* (maintained on leeks). Kindly supplied by Dr. M.J. Daft, Dundee University.

V	*Glomus mosseae*	maintained on onion	Kindly supplied by Dr F.E. Sanders, Leeds University
VI	*Glomus macrocarpus*		
VII	*Glomus clarus*		

The inoculum was sand containing spores, infected root segments and fragments of hyphae. Plants which were not inoculated with mycorrhiza received a leachate containing micro-organisms, other than mycorrhizal fungi, present in the inoculum (Hayman and Mosse, 1971).

Measurements

In addition to the measurements described in Part I, mycorrhizal infection of roots was assessed. Roots were cleared with 2N KOH at room temperature for 2-7 days, washed with water and stained with 0.01% trypan blue in lactic acid. Approximately 35 root segments were placed

in parallel on a microscope slide; three transects of the roots were scanned under the compound microscope and the number of infected root segments in each transect were counted. Two to three slides were made from roots from each pot.

Replication and experimental design

There were five replicated blocks in all experiments except Experiment 10, the details for which are given in the experimental section. All treatments were completely randomised within the blocks (with the exception of Experiment 10).

Statistical analysis

Where necessary, the data were transformed before analysis and the transformations used were $\log_e (x)$, and $\log_e (x + 1)$. Significant differences between values after analysis of the transformed data are indicated by a different letter after the value.

FIELD EXPERIMENTS

The experiments were set up at the three sites from which soil was collected for the pot experiments.

Treatments

The mycorrhizal inoculants, fertilizers applied and cultivars used in the three field trials are listed in Table 35.

Preparation of the sites

The indigenous vegetation was cut from the peat site; the brown earth site at Sourhope was rotovated twice and

Table 35. The fertilizers, inoculants and cultivars used in the mycorrhizal field trials

Experiment	Fertilizers applied (kg/ha) N	P as single superphosphate	K as muriate of potash	Trace elements soln. prepared as on page 45	Lime/Mg as magnesian limestone	Inoculant	Cultivar
14	None	0 20 200	160	applied	7000	G. mosseae L1	Huia S184
15	None	0 40	40	applied	5000	G. mosseae L1 G. caledonius	Huia
16	None	0 50	100	applied	4000	G. mosseae L1 G. mosseae G. clarus G. macrocarpus	S184

rolled; and the brown earth at Cleish was ploughed, rotovated and rolled.

Lime was applied in early spring but potassium, superphosphate and trace elements were applied to plots about a week before sowing. White clover seed was inoculated with rhizobia by the peat sticker technique (Vincent, 1970) the day before the seed was sown (the seed rate was 5 kg/ha). At Lephinmore, the seed and inoculum were broadcast and the material was raked into the surface mat. At Sourhope, the seed and inoculum were also broadcast but raking was easier in the rotovated soil and, at Cleish, the seed and inoculum was drilled in the soil.

Replication and experimental design

There were three replicated blocks in the Lephinmore experiment and four replicated blocks in the experiments at Sourhope and Cleish. In all the experiments the treatments were completely randomised within the blocks.

PLAN OF THE EXPERIMENTS

Nine experiments are described in the next section. The first six are laboratory experiments (Experiments 8-13) and the remaining three are field trials (Experiments 14-16). Of the pot experiments, the first two examine in some detail the responses to phosphorus and potassium by mycorrhizal and non-mycorrhizal plants grown in the deep peat. The third attempts to quantify the density of propagules of indigenous endophytes in the three soils

and the last three laboratory experiments screen the effects of a number of endophytes on clover grown in the soils collected from the field sites.

THE EXPERIMENTS

The seedlings in the Lephinmore peat without added phosphorus, in Experiment 1, initially grew very slowly and looked as though they would die but gradually, after 3-4 weeks, they became green and healthy although they remained relatively small; examination of the roots indicated that they were infected with VA mycorrhizal fungi. Accordingly, an experiment was set up to investigate the possible role of indigenous mycorrhizal fungi in the phosphorus nutrition and early establishment of white clover in this soil.

EXPERIMENT 8. Inoculation of white clover with indigenous endophytes in non-sterile peat with two levels of added phosphorus

EXPERIMENTAL

The inoculum was prepared by growing maize in sterile sand which contained the chopped roots from plants from Experiment 1 described above. After 12 weeks the maize was harvested and the yellow roots chopped into small segments and used to inoculate white clover grown in peat in the pot experiment.

Two levels of phosphorus, 40 and 160 kg P/ha were applied as $Ca_3(PO_4)_2$, with basal dressings of 2.9 tonnes lime/ha, 160 kg K/ha and trace elements. The inoculum was placed in a layer in the peat, 1 cm below the seed. After germination the seedlings were inoculated with

rhizobia. The plants were grown in the glasshouse for 47 days from 6 August 1976 to 21 September 1976.

RESULTS

Levels of infection (Table 36)

Inoculation increased the amount of infection of the root from 13 and 3% to 54 and 47% with 40 and 160 kg P/ha respectively. Both levels of phosphorus tended to reduce the percentage of infected root.

Yield of clover (Table 36, Fig. 25)

The greatest response to inoculation was at the lower level of phosphorus where there was a 1.6 fold increase in total yield. However, there was a 0.2 fold increase in yield when clover was inoculated at the higher rate of phosphorus. Inoculated plants given 40 kg P/ha made as much growth as uninoculated plants given 160 kg P/ha.

The response by the root was of a similar magnitude to that of the shoot.

The nutrient content of the shoot (Table 37)

(a) Concentration. The concentration of phosphorus, calcium and magnesium in the shoot was greater and the concentration of potassium in the shoot was less with 160 kg P/ha than with 40 kg P/ha. There was no effect of inoculation on the concentration of nutrients at the greater level of added phosphorus but, at the lower level of added phosphorus, inoculation reduced the

Table 36. The effect of inoculation with indigenous endophytes on the level of root infection and yield of white clover in non-sterile peat with two levels of added tricalcium phosphate (Experiment 8)

	Phosphorus (kg/ha)				
	40		160		
	Inoculant				SED
	None	Indigenous	None	Indigenous	(12 df)
Root infection (%)	13	54	3	47	5.0
Yield (g DM/pot)					
Shoot	0.7	1.8	2.0	2.3	0.11
Root	0.3	0.8	0.7	0.9	0.06
Total	1.0	2.6	2.7	3.2	0.16

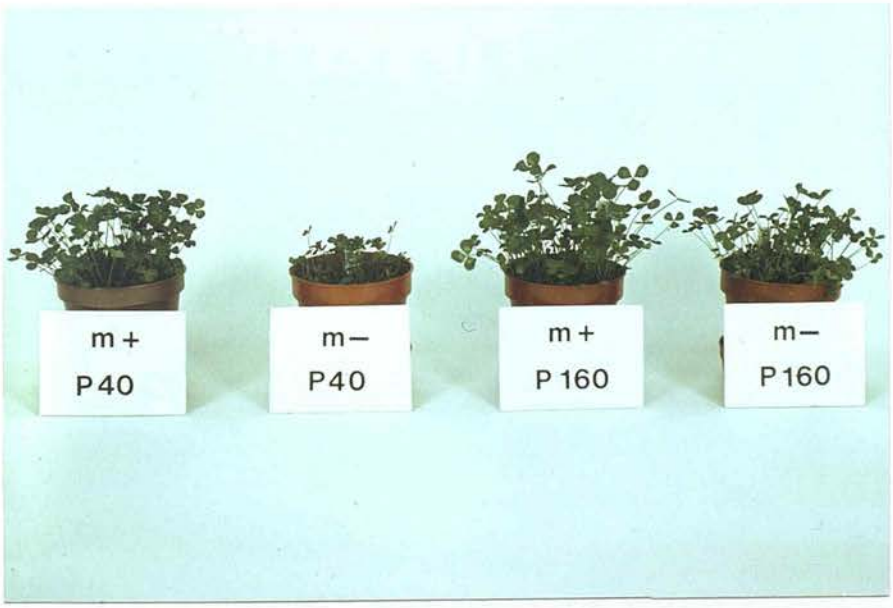


Figure 25. White clover inoculated with indigenous endophytes and given two levels of tricalcium phosphate in Lephinmore deep peat (Experiment 8)

Table 37. The effect of inoculation with indigenous endophytes on the nutrient content of the shoots of white clover grown in non-sterile peat with two levels of added tricalcium phosphate (Experiment 8)

	Phosphorus (kg/ha)				
	40		160		
	Inoculant				SED
	None	Indigenous	None	Indigenous	(12 df)
Phosphorus					
Concentration (%)	0.29	0.22	0.38	0.41	0.019
Uptake (mg/pot)	2	4	8	9	0.6
Potassium					
Concentration (%)	2.68	1.03	0.92	0.81	0.18
Uptake (mg/pot)	19	18	18	19	1.1
Calcium					
Concentration (%)	2.93	2.45	3.50	3.37	0.20
Uptake (mg/pot)	21	44	68	77	3.9
Magnesium					
Concentration (%)	0.38	0.33	0.49	0.48	0.017
Uptake (mg/pot)	3	6	10	11	0.4

concentration of all the nutrients measured.

(b) Uptake. Inoculation greatly increased the amount of phosphorus, calcium and magnesium in the shoot at the lower level of phosphorus and, at the higher level of phosphorus, the uptake of calcium and magnesium was just significantly increased. The uptake of potassium was unaffected by treatment.

Nodulation and nitrogen fixation (Table 38)

Inoculation had the greatest effect on nodulation and nitrogen fixation at the lower level of added phosphorus.

There were fewer nodules on plants roots which received 40 kg P/ha than on roots grown in peat with 160 kg P/ha even after inoculation. However, the ratio of small:large nodules was the same on inoculated plants with 40 kg P/ha as with 160 kg P/ha.

The total number of nodules per unit weight of root was about the same on inoculated and uninoculated plants at 40 kg P/ha but inoculation greatly increased the number of nodules longer than 1 mm. With 160 kg P/ha the number of small nodules per gram of root was slightly reduced.

The effect of inoculation on the activity of nitrogenase in the nodules (measured by acetylene reduction assay) and the amount of nitrogen in the shoots was similar to the effect on nodulation.

The concentration of nitrogen in the shoots was very high (5.4% N) in uninoculated plants given 40 kg P/ha

Table 38. The effect of inoculation with indigenous endophytes on the number of nodules, the acetylene reduced the day before harvest and the nitrogen content of the shoot of white clover grown in non-sterile peat with two levels of added tricalcium phosphate (Experiment 8)

	Phosphorus (kg/ha)				
	40		160		
	Inoculant				SED
	None	Indigenous	None	Indigenous	(12 df)
Number of nodules per pot					
<1 mm	204	375	637	711	40.3
>1 mm	14	131	191	225	19.7
Ratio <1 mm: >1mm	15	3	3	3	
Number of nodules per gram of root					
<1 mm	658	463	951	826	36.8
>1 mm	45	162	285	262	40.9
Total	703	625	1236	1088	45.7
Acetylene reduction (μ moles/pot/hour)	2	19	33	34	3.0
Nitrogen content of shoot					
concentration (%)	5.4	3.9	4.0	3.6	0.28
amount (mg/pot)	39	69	79	82	4.5

compared with the other treatments (3.6-4.0% N) and suggested that growth was restricted by some element other than nitrogen.

The effect of mycorrhiza on the growth of white clover in this experiment was measured over a short period of time (47 days) during the establishment of the plant. However, white clover is a perennial crop and possible benefits from mycorrhizas in the established pasture are of great interest and importance, as are interactions with the other major nutrients applied as fertilizers. Experiments 1-4 in Part I of the present work emphasise the importance of the balance between applications of phosphorus and potassium for the most efficient use of the fertilizers and several workers (Smith and Daft, 1977; Smith et al., 1979) have suggested that mycorrhizas may have an additional effect on nodulation and nitrogen fixation which is not accounted for by the improved phosphorus nutrition of the host. Accordingly, a pot experiment was set up to measure the longer term effects of mycorrhizas on the growth of clover when relying for nitrogen almost entirely on either mineral N or biologically fixed N. In addition, the plants were given the four combinations from two levels of applied phosphorus and two levels of applied potassium.

EXPERIMENT 9. The effect of G. mosseae (L1) form of nitrogen, phosphorus and potassium fertilizers on the growth of white clover in peat

EXPERIMENTAL

Some of the results from this experiment, the effects of phosphorus and potassium on the growth of leaves, were given in Part I, Experiment 2. Here the effects of mycorrhizas on the growth of white clover and nitrogenase activity in the nodules for successive harvests over the 193 days from sowing are reported. In addition, the effect of mycorrhizas on flowering is given.

The details of how the experiment was set up were described in Experiment 2, p.76. Briefly, 10 plants were grown in the Lephinmore peat in 7.5 cm diameter plant pots in a growth room. The phosphorus and potassium treatments were:-

No P	:	No K
40 kg P/ha	:	40 kg K/ha
40 kg P/ha	:	160 kg P/ha
160 kg P/ha	:	40 kg K/ha
160 kg P/ha	:	160 kg K/ha

Superimposed on the phosphorus and potassium treatments were mycorrhizal and nitrogen treatments. Plants were inoculated with G. mosseae L1 at sowing and the inoculum of infected sand (10 g/pot) was placed 1 cm below the level of the seed in the pot. Uninoculated plants received an equal amount of autoclaved sand and leachates from the mycorrhizal inoculum. Uninoculated plants in the previous experiment were poorly infected and it was assumed that the same was true in this experiment although the roots were not examined for

infection. Therefore, the comparison between inoculated and uninoculated plants was essentially one between mycorrhizal and non-mycorrhizal plants.

Plants were either dependent for nitrogen on biological fixation and mineralisation of soil nitrogen, or were watered daily with mineral nitrogen (200 ppm N as ammonium nitrate), incorporated into the trace element solution which was applied to all the pots (see p. 45).

Therefore, there were four treatments superimposed:-

non-mycorrhizal with fixed nitrogen

non-mycorrhizal with mineral nitrogen

mycorrhizal with fixed nitrogen

mycorrhizal with mineral nitrogen

Plants were defoliated and deflowered eight times over a period of 193 days after sowing. Each harvest took 5 days; one replicate was harvested on each day and began on days 35, 48, 62, 76, 90, 123, 151 and 193 after sowing. The pots from each replicate were assayed for nitrogenase activity of the nodules using the acetylene reduction technique (Dart, Day and Harris, 1972) from 9.00 a.m. to 12 noon, and the shoots were harvested in the afternoon.

The information presented is averaged over several treatments and information for individual treatments is given in Appendix 13, the yield of green leaves; Appendix 14, acetylene reduced (μM/10 pl/hr); and Appendix 15, flower number.

RESULTS

The nitrogen treatment (Table 39)

Plants grew better when given combined nitrogen (200 ppm ammonium nitrate solution) than when grown on fixed nitrogen at harvests 1 and 2 but, from harvests 5 to 8, growth was on average less (Table 39). The effect of combined nitrogen was greater in the early harvests, when the higher levels of phosphorus and potassium were applied but, after harvest 4, growth was more severely depressed with the lower levels of phosphorus and potassium. The nitrogen was probably toxic when other nutrients were limiting growth.

The plants given mineral nitrogen did not reduce any acetylene at any of the sampling times. There were no interactions between the growth of plants given combined nitrogen or fixed nitrogen and the phosphorus or potassium fertilizer treatments. Nor was there an interaction between the nitrogen source and mycorrhiza except from 0-35 days (Fig. 26). The effects of mycorrhiza, phosphorus and potassium on clover growth will therefore be discussed for plants deriving their nitrogen from symbiotic nitrogen fixation but the results are applicable to plants given mineral nitrogen.

The cumulative yield of green leaves up to 193 days from sowing (Fig. 27)

(a) Uninoculated plants. There was little growth (<0.05 g DM/pot) in the absence of added phosphorus and potassium and most growth (3.7 g DM/pot) was made by

Table 39. The effect of application of a nitrogen solution (200 ppm N) on the rate of growth of green leaves (mg DM/10 day) of white clover (Experiment 9)

Growth period	Fixed nitrogen	Mineral nitrogen	SED (76 df)
0-35	12.5	17.3	0.9
35-48	32.2	46.1	3.5
48-62	27.9	32.8	3.0
62-76	26.1	22.1	2.1
76-90	26.1	15.2	1.6
90-123	7.2	3.4	0.6
123-151	7.1	2.8	0.7
151-193	0.6	0.2	0.1

Table 40. The effect of G.mosseae L1 on the flower number of white clover (No/pot) (Experiment 9)

Growth period	Uninoculated	Inoculated	SED (76 df)
0-35	0	0	-
35-48	1.4	0.6	0.35
48-62	2.4	2.7	0.47
62-76	0.5	0.8	0.18
76-90	0.3	0.1	0.10
90-123	0.2	0.2	0.17
123-151	0.1	0.1	0.06
151-199	0.1	0.2	0.08

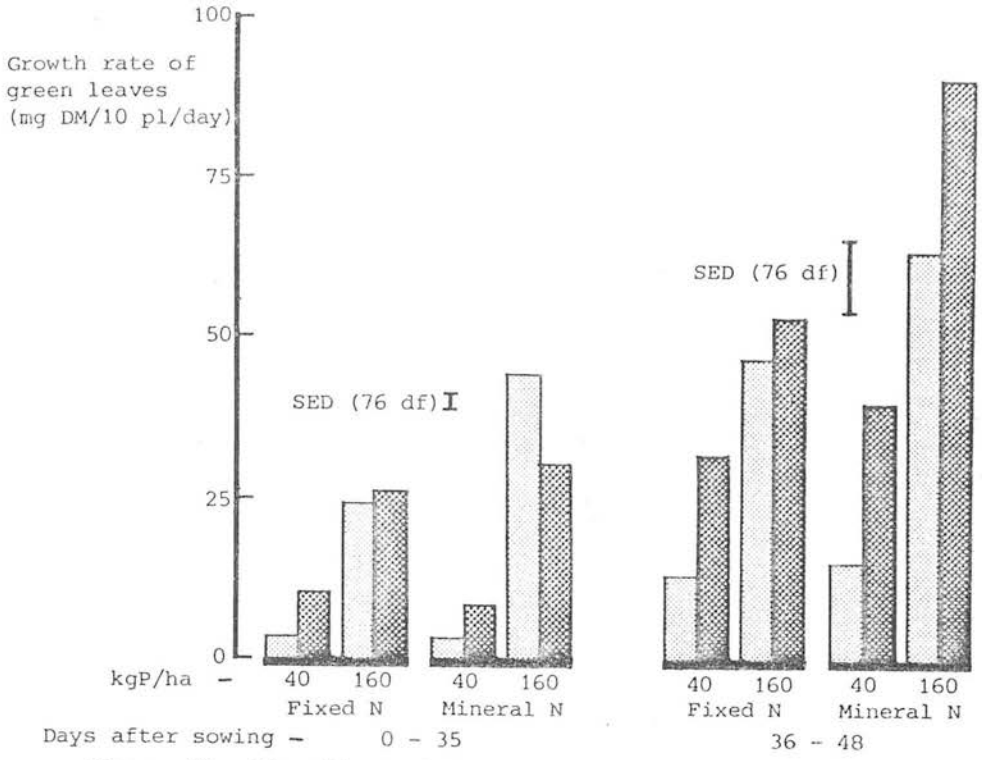


Figure 26. The effect of *G. mosseae* (L1), source of nitrogen and level of application of phosphorus on the rate of growth of green leaves (mgDM/10pl/day) of white clover during two periods of time (Experiment 9).

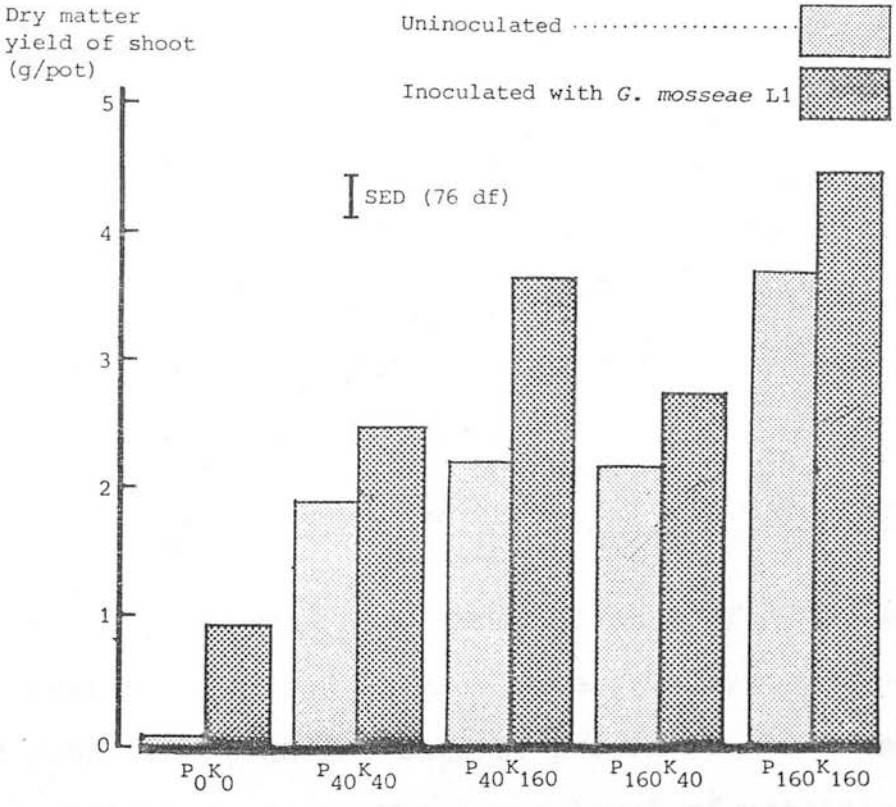


Figure 27. The effect of *G. mosseae* (L1) and added phosphorus and potassium on the cumulative yield (0 to 193 days from sowing) of green leaves of white clover (Experiment 9)

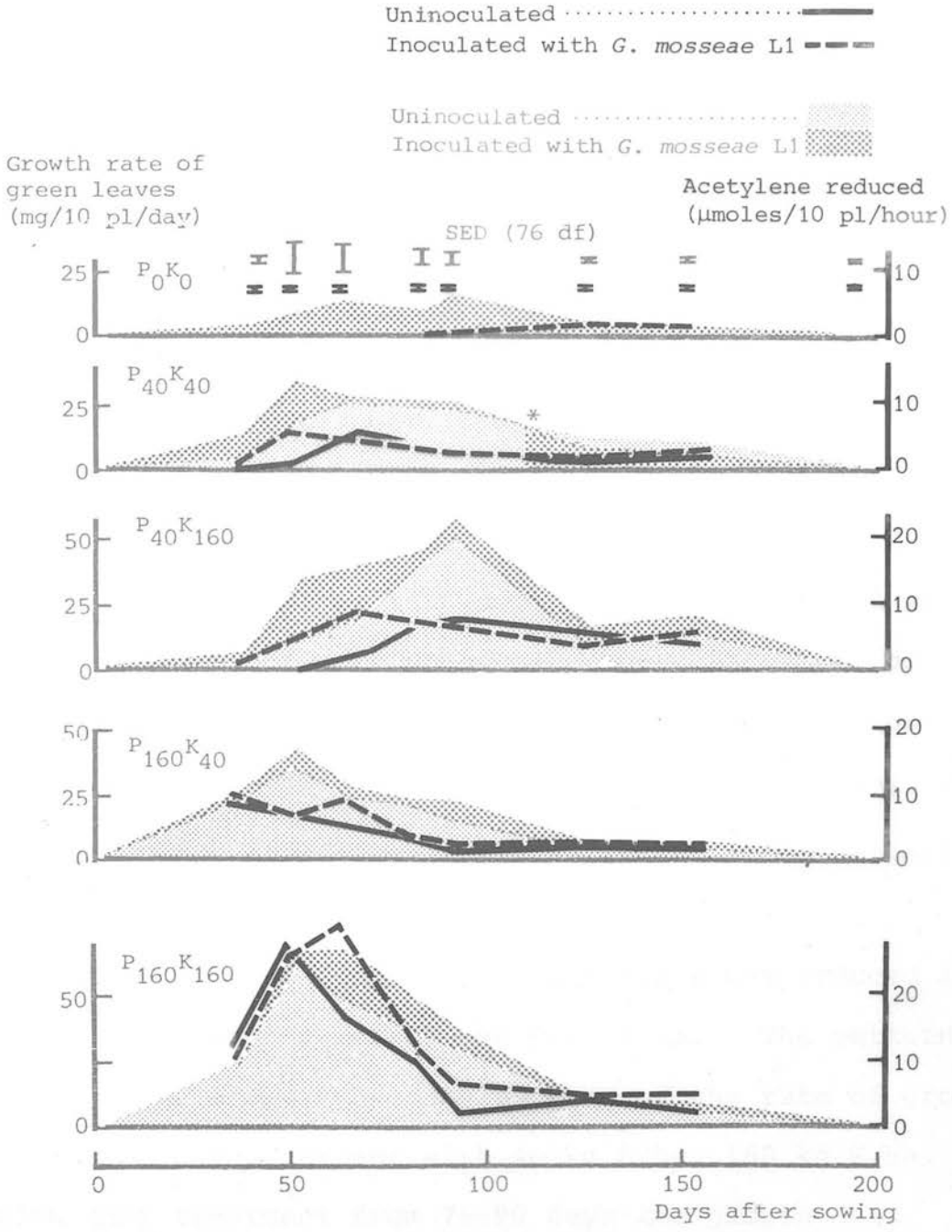
plants given 160 kg P/ha with 160 kg K/ha. Growth with other fertilizer treatments, when uninoculated, was similar (about 2 g DM/pot).

(b) Inoculated plants. Nearly 1 g DM/pot was produced in the absence of added phosphorus and potassium when plants were inoculated with L1 whereas plants produced 4.5 g DM/pot when 160 kg P/ha and 160 kg K/ha was applied. With both levels of phosphorus, given 40 kg K/ha, growth was similar (about 2.5 g DM/pot), but with 40 kg P/ha and 160 kg K/ha, growth approached 3.7 g DM/pot.

Inoculation with L1 resulted in the greatest stimulation of growth (34 fold) in the absence of phosphorus and potassium. With 40 kg P/ha the effect of inoculation depended upon the level of potassium applied; with 40 kg K/ha there was a 0.3 fold increase in growth and, with 160 kg K/ha there was a 0.6 fold increase in growth. Growth of plants given 160 kg P/ha was stimulated at both levels of potassium by about the same amount, 0.26 and 0.21 fold.

The growth rate of green leaves during 193 days after sowing (Fig. 28)

(a) Uninoculated plants. Growth rates of green leaves were greatest with 160 kg P/ha after 50 days, but with 40 kg P/ha, the greatest rate of growth occurred later, 90 days after sowing. The lower level of potassium (40 kg K/ha) at both levels of phosphorus reduced the growth rate compared with plants given the greater level



* Growth rate of mycorrhizal plants becomes less than non-mycorrhizal plants.

Figure 28. The effect of *G. mosseae* (L1) and added phosphorus and potassium on the rate of growth of green leaves (mgDM/10pl/day) of white clover during the 193 days after sowing (Experiment 9).

The overlay gives the rates of nitrogenase activity measured by acetylene reduction.

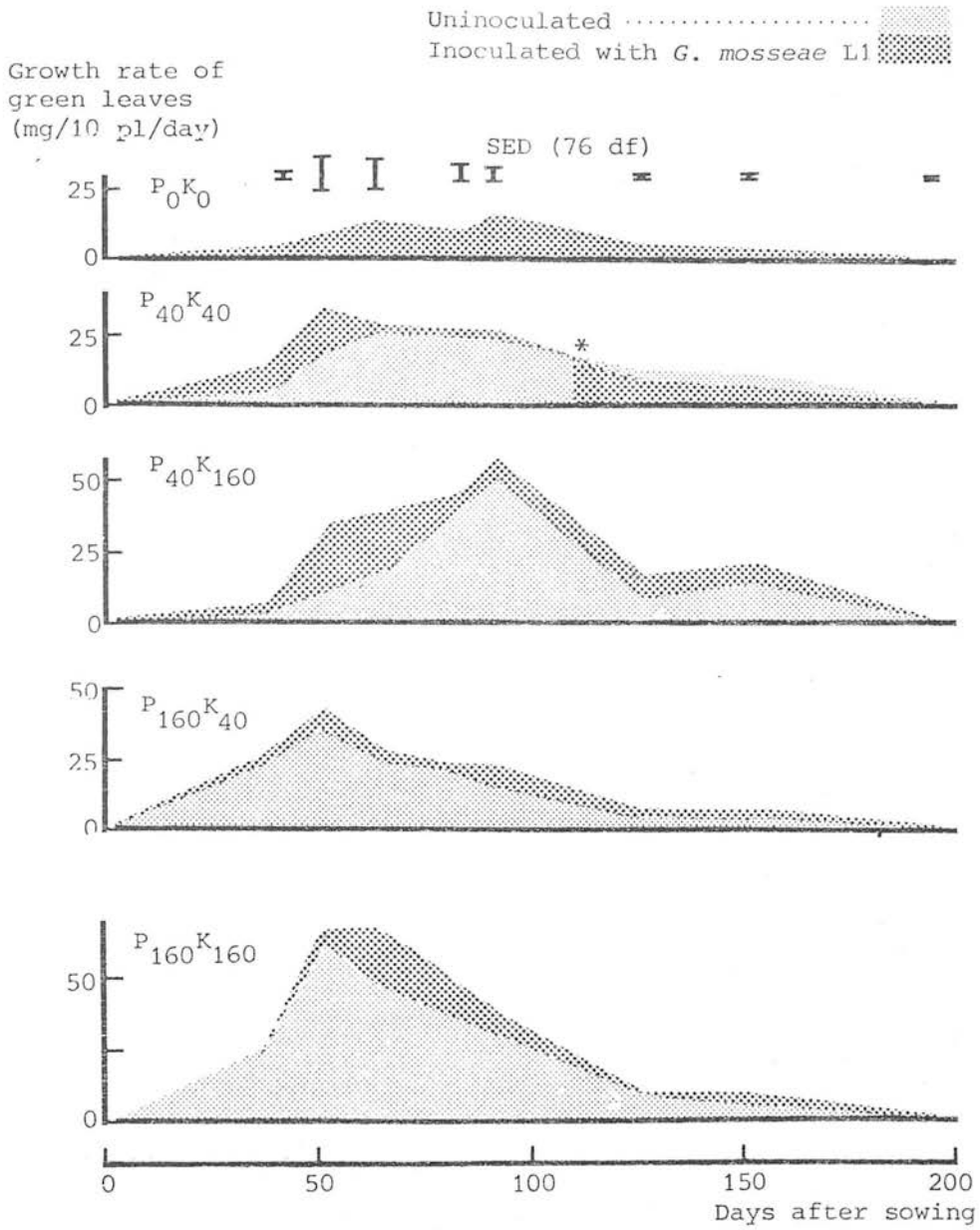


Figure 28. The effect of *G. mosseae* (L1) and added phosphorus and potassium on the rate of growth of green leaves (mgDM/10pl/day) of white clover during the 193 days after sowing (Experiment 9).

of potassium (160 kg K/ha) except with 40 kg P/ha from 35-64 days.

(b) Inoculated plants. The effect of mycorrhizal inoculation varied with fertilizer treatment and the general pattern of response is described although the effects are not all significant. With 40 kg P/ha mycorrhiza were most beneficial during the early growth of the plant, up to day 62 with the lower level of applied potassium (40 kg K/ha), and up to day 90 with the higher level of potassium. With 160 kg P/ha, mycorrhiza had little effect on growth with the lower level of potassium and the beneficial effects on growth when plants were given high levels of both phosphorus and potassium did not develop until after day 48, but continued until day 90.

Nitrogenase activity (measured by acetylene reduction) Fig. 28 overlay)

(a) Uninoculated plants. No acetylene was reduced in the absence of phosphorus and potassium. The pattern of nitrogenase activity resembled that of the rate of growth of green leaves, except with 40 kg P/ha, 160 kg K/ha. With this treatment from 76-90 days the growth rate increased but was not accompanied by a corresponding increase in nitrogenase activity.

(b) Inoculated plants. The beneficial effect of mycorrhiza on nitrogenase activity was similar to the effect on the rate of growth of green leaves except for the treatment 40 kg P/ha, 160 kg K/ha. With this treatment there was a beneficial effect of mycorrhiza on the

growth rate for the whole period measured (193 days) but there was a greater rate of nitrogenase activity only from 35-76 days.

Number of flowers (Table 40)

During the experiment the plants flowered and the effects of inoculation were measured. Possible hormonal effects of mycorrhiza on plant growth are alluded to in the literature. Therefore, mycorrhiza may affect flowering as it is under hormonal control in the plant. However, no effects of mycorrhiza on flowering were found, except for a delay between days 35-48.

Therefore, the results from this experiment and the previous one (Experiments 8 and 9) with information from the literature (e.g. Powell, 1976a; Sparling and Tinker, 1975; Crush, 1974) have suggested that inoculation of white clover with mycorrhizal fungi in the field may enhance early growth when low levels of phosphorus fertilizer are applied, or may be beneficial in the later growth of pasture with higher levels of phosphorus fertilizer. A series of three field trials were undertaken at three sites, the objective of which was to demonstrate increased yields from clover inoculated with mycorrhizal fungi. Dr. M.J. Daft (Dundee University) agreed to collaborate. He principally supplied the inocula and measured levels of root infection while the author set up the sites and did the harvests. The treatments applied were decided jointly. In addition

to the inocula provided by Dr. Daft, Dr. F.E. Sanders (Leeds University) provided inocula for use in the field experiment at Cleish (Experiment 16).

In conjunction with the field trials, three laboratory experiments tested the effectiveness of the inocula in the three soils, and one experiment measured the density of propagules of indigenous endophytes in the soils. This latter experiment will be described first, followed by the pot experiments with the three soils and then by the field trials.

EXPERIMENT 10. Indigenous endophytes in three soils

EXPERIMENTAL

In the past, the presence of endophytes in a soil was assessed by counting the number of spores present, but there is increasing evidence that the methods used to separate spores from soils are inaccurate (Walker, 1979) and that some mycorrhizal fungi do not sporulate (Baylis, 1969; Owusu-Bennoah and Mosse, 1979). In this investigation an attempt was made to count the spores in the soils but problems encountered in sieving the organic soils and inexperience at identification made the results worthless. Instead, an attempt was made to count all the propagules by setting up a dilution series of the soils with sterile sand and recording the presence or absence of infection.

Little is known about the effect of age on the infectivity of mycorrhizal propagules, but Johnson (1977)

found that infected roots from plants decapitated three months before use were less infective than roots from plants decapitated just before the experiment. To allow a valid comparison between soils in the experiment, the soils were collected within three days of each other and were kept at 4^{0}C for two weeks before the seeds were sown. The soil samples were also collected from the same depth; the turf was skimmed from the surface and the samples taken from 5-10 cm depth thereby minimising possible complications due to uneven vertical distribution of propagules in the soil.

Ten grams (dry weight) of each soil were taken and the soil was mixed with autoclaved sand until the volume was sufficient to fill four plastic tubes. The sand and soil were thoroughly mixed and three tubes were filled with the mixture. To the remaining volume, a further three volumes of sand were added and mixed. There were five dilutions which yielded three tubes per dilution and there were three runs with each soil and therefore there were nine tubes per dilution of one soil. Each dilution series contained tubes with 2.5 g, 625 mg, 156 mg, 39 mg and 10 mg of soil mixed evenly throughout sterile sand.

The plastic tubes used were 1 cm diameter and 7 cm long. A small hole was made in the bottom of each tube to allow drainage and the tubes were painted black. Each run (1 soil x 5 dilutions x 3 tubes) was placed together in a test tube rack and the runs and soils were

completely randomised and placed in the growth chamber.

Three seeds were sown into the soil/sand mixture and were thoroughly watered with half strength Dart and Pate nutrient solution (Dart and Pate, 1959). After germination the seedlings were thinned to one per tube. To prevent contamination, glass vials were placed over the top of the tubes and secured with PVC tape. The seedlings were watered with half strength Dart and Pate nutrient solution daily taking care that each vial was removed singly. Removal of the vial also allowed gas exchange. A photograph of the tubes in the growth chamber is shown in Fig. 29 which shows sufficient tubes for four soils but the results for the fourth soil are not presented here.

The seedlings were harvested one month after sowing and the presence or absence of mycorrhizal infections were noted.

RESULTS (Table 41)

The density of mycorrhizal propagules in the soils varied. There were comparatively few in the Lephinmore peat, less than one in 2.5 g soil which, on a volume basis (0.1 mg soil/cm^3), represents less than one per 25 cm^3 in the field. In the two brown earth soils, all plants were infected from 625 mg soil and therefore there was at least one propagule per cm^2 of soil (density approximately 0.6-0.7 g/cm^3). The data suggest that there was one propagule per cm^3 (22% of plants with 156 mg of soil were infected) in the Sourhope brown earth and there

Table 41. The percentage of plants infected with mycorrhizal fungi when grown in different amounts of soil (diluted to a constant volume with sterile acid washed sand (Experiment 10)

Soil	Dry weight of soil 2.5g	625mg	156mg	39mg	10mg
Lephinmore deep peat	50	0	0	0	0
Sourhope brown earth	100	100	22	0	0
Cleish brown earth	100	100	78	0	0

Figure 29. Seedlings in tubes in the growth room during the soil dilution experiment (Experiment 10)

were three propagules per cm^3 (78% of plants were infected with 156 mg soil) of the Cleish brown earth.

EXPERIMENT 11. The effect of G. mosseae (L1) and G. fasciculatus (E3) and gafsa phosphate on the growth of two cultivars of white clover in peat

EXPERIMENTAL

The experimentation in the following pot experiment differs from that previously described and some explanation is required. In July 1976 a group of workers from the UK, interested in mycorrhizal associations in hill pastures, with particular reference to white clover, met for discussions. At that meeting it was recommended that field trials in different parts of the country, carried out by different workers, should include the cultivar of white clover S184 inoculated with the mycorrhizal fungus, G. fasciculatus as a standard for comparison. However, the inoculant in plentiful supply at Dundee was G. mosseae L1.

At the same meeting Dr. D. Hayman (Rothamsted Experimental Station) described a pot experiment where white clover cv. Huia inoculated with E3 had been grown in Lephinmore peat. The results were contrary to those obtained in Experiment 9. The experimental techniques in the two experiments were different and it was agreed that each experiment should be repeated at the other institute.

Accordingly, the effects of G. fasciculatus and G. mosseae L1 were compared on two cultivars of white clover, Huia and S184 in the deep peat. Seeds were sown in sterile sand or sand infected with the endophytes. After 4 weeks, when the seedlings had the first trifoliate leaf and were well infected with the mycorrhizal fungi, one seedling was transplanted into each pot of Lephinmore peat that had been limed (2.7 tonnes/ha) and given 59 kg K/ha with or without gafsa phosphate at 13 kg P/ha. The seedlings were inoculated with rhizobia when they were transplanted, in addition to the standard inoculation one week after germination, and were given trace elements. The plants were grown in the growth room and were harvested 60 days after they were transplanted.

RESULTS

Levels of root infection (Table 42)

Infection of uninoculated Huia in the absence of gafsa (1% of the root) was less than that of uninoculated S184 (18% of the root). Inoculation increased root infection levels from 1-18% to 84-95%. There were no differential effects of the endophytes on the cultivars.

Yield of the shoots (Table 42)

Inoculation increased growth from 11 mg dry matter/pot or less to 169 mg dry matter/pot or more. S184 responded to gafsa but Huia did not. There were no interactions between endophytes and cultivars.

Table 42. The effect of Glomus mosseae (L1), G. fasciculatus (E3) and gafsa phosphate on the dry weight of shoots and the root infection of two cultivars of white clover grown in non-sterile peat (Experiment 11)

Cultivar	Gafsa (kg P/ha)	Inoculant	Shoot dry weight (mg/pot)	Root infection (%)
S184	0	None	8 a†	1 x†
		L1	169 b	86 z
		E3	267 b	84 z
	13	None	7 a	11 xy
		L1	490 b	87 z
		E3	467 b	93 z
Huia	0	None	3 a	18 y
		L1	289 b	89 z
		E3	246 b	95 z
	13	None	11 a	17 y
		L1	265 b	89 z
		E3	317 b	87 z

† The data was transformed to Log_e (x + 1) for statistical analysis. Values are significantly different when the letter after the number is different

EXPERIMENT 12. The effect of G. mosseae (L1) and gafsa phosphate on the growth of white clover in the brown earth from Sourhope

EXPERIMENTAL

The experiment was set up using the same techniques as in the previous experiment. Less lime was applied (0.7tonnes $CaCo_3$/ha) and, because there were no cultivar x endophyte interactions in the previous experiment, only the effect of L1 on Huia was measured. The experiment was harvested three times; the expanded leaves and flowers were removed and dried at harvests 1 and 2 and, at harvest 3, the total shoot was dried and the roots were washed, cleared and stained for measurement of infection.

RESULTS

Levels of root infection (Table 43)

Infection of roots was relatively high (63 to 77%) in uninoculated plants. Inoculation increased total infection only when gafsa was applied. The indigenous endophytes in this soil mostly had fine hyphae and it was possible to measure the increased infection caused by inoculation with a relatively coarse endophyte. Inoculation doubled infection caused by coarse endophytes from 25 to 29% to 59 to 61%, but only reduced infection by fine endophytes in the absence of gafsa from 68 to 47%. Infection by one endophyte can rise without affecting the measured amount of infection by another because both endophytes can infect the same section of root.

Table 43. The effect of Glomus mosseae (L1) and gafsa phosphate on the dry weight of the shoots at three harvests 20, 40 and 60 days after transplanting and the root infection in white clover cv Huia grown in the Sourhope brown earth soil (Experiment 12)

Cultivar	Gafsa (kg P/ha)	Inoculant	Shoot dry weight (g/pot) H1	H2	H3	Root infection at harvest 3 (%) Fine	Coarse	Total†
Huia	0	None	0.8	1.5	2.7	68	29	77
		L1	0.9	1.4	2.3	47	61	79
	13	None	0.7	1.5	2.5	47	25	63
		L1	0.7	1.5	2.3	51	59	85
SED (12 df)			0.11	0.16	0.41	6.1	11.5	6.7

† The sum of the fine and coarse root infection can be more than the total infection because both endophytes can occur in the same measured section of root

Yield of the shoots (Table 43)

There was no effect of inoculation or gafsa on the growth of clover at harvest 1 so it was decided to take more harvests to see if growth differences developed later. They did not.

EXPERIMENT 13. The effect of four endophytes and superphosphate on the growth of white clover in the brown earth from Cleish

EXPERIMENTAL

Four endophytes, G. mosseae (L1), G. mosseae, G. macrocarpus and G. clarus, were inoculated onto white clover cv. S184. A basal dressing of 4 tonnes lime/ha and 100 kg K/ha was applied with none or 50 kg P/ha as superphosphate. The inoculum (10 g/pot) was placed in the soil in a layer 1 cm below the level of the seed. Forty seeds were sown in each 10 cm diameter pot. Trace elements and rhizobia were given. The experiment was sown 18 October 1979 and harvested on 18 December 1979, 61 days after sowing.

RESULTS

Levels of root infection (Table 44)

The level of root infection varied from 53 to 80%. The level of infection in uninoculated plants was similar to the infection in clover roots inoculated with three of the four endophytes. However, inoculation with L1

Table 44. The effect of four mycorrhizal inoculants and superphosphate on the dry weight of the shoots and root infection of white clover grown in the Cleish brown earth (Experiment 13)

Cultivar	Superphosphate (kg P/ha)	Inoculant	Shoot dry weight (g/pot)	Root infection (%)
S184	0	None	0.7	70
		G. mosseae L1	0.8	53
		G. mosseae	0.7	73
		G. macrocarpus	0.7	77
		G. clarus	0.7	76
	50	None	2.3	71
		G. mosseae L1	2.3	54
		G. mosseae	2.2	80
		G. macrocarpus	2.2	62
		G. clarus	2.3	63
SED (36 df)			0.09	5.3

reduced the level of infection. Superphosphate was without effect.

Yield of the shoots (Table 44)

The inoculants had no effect on growth but yield of the shoot was three times greater when superphosphate was applied.

The three field experiments in the Lephinmore peat, the Sourhope brown earth and the Cleish brown earth follow in turn.

EXPERIMENT 14. The effect of G. mosseae (L1) and superphosphate on the growth of two cultivars of white clover in the deep peat at Lephinmore

EXPERIMENTAL

The effect of G. mosseae (L1) and three levels of superphosphate (0, 20 and 200 kg P/ha) on the growth of two cultivars of white clover, Huia and S184, was compared in the field at Lephinmore. The indigenous vegetation was cut and removed but the soil was too wet for cultivation and 2 x 4 m plots were pegged out. Basal dressings of 7.5 tonnes lime/ha and 80 kg K/ha were applied with trace elements and the seed was coated with rhizobia. The inoculum (4 m^3/ha) and seed (5kg/ha) were broadcast and raked into the surface mat on the soil. The experiment was sown in August 1977 and harvested in August 1978.

Table 45. The effect of Glomus mosseae (L1) and superphosphate on the dry matter yield and root infection of two cultivars of white clover grown at Lephinmore on a deep peat (Experiment 14)

Cultivar	Superphosphate (kg P/ha)	Inoculant	Dry matter yield (kg/ha)	Infected root (%)
S184	0	None	3 a†	13
		L1	<1 a	39
	20	None	373 de	15
		L1	287 cd	36
	200	None	862 e	15
		L1	877 e	38
Huia	0	None	<1 a	16
		L1	1 a	53
	20	None	129 bc	37
		L1	116 b	52
	200	None	122 bc	15
		L1	117 b	34
SED (22 df)				6.3

† The data was transformed to $\text{Log}_e(x + 1)$ for statistical analysis. Values are significantly different when the letter after the number is different

RESULTS

Levels of root infection (Table 45)

In all but one treatment 13-16% of the roots of uninoculated plants were mycorrhizal; the roots of Huia with 20 kg P/ha had 37% infection. Inoculation with L1 more than doubled the level of infection. There were no interactions between cultivar and L1.

Dry matter yield (Table 45)

Inoculation had no effect on dry matter yield of white clover. Very few plants grew in the absence of phosphorus; Huia responded to the lower level of phosphorus but not thereafter; S184 responded to both levels of phosphorus.

EXPERIMENT 15. The effect of G. mosseae (L1), G. caledonius and superphosphate on the growth of white clover in a brown earth at Sourhope

EXPERIMENTAL

The effect of G. mosseae L1, G. caledonius and superphosphate on the growth of white clover cv. Huia was measured in the field on a brown earth at Sourhope. Basal dressings of 5 tonnes lime, 100 kg K/ha and trace elements were applied. The soil was rotovated and rolled, and 2 x 2 m plots were pegged out. The seed (sown at 5 kg/ha and inoculated with rhizobia) was broadcast onto the plots together with mycorrhiza inoculum (4 m^3/ha) and raked into the surface on 25 April 1978. The herbage was sampled in October 1978, July 1979 and October 1979. Annual dry

matter yields for 1978 and 1979 are presented and data for the two cuts in 1979 are given in Appendix 16. Roots were sampled in October 1978 and December 1979.

RESULTS

Levels of root infection (Tables 46 and 47)

In 1978 the level of infection by indigenous endophytes was high (76-83% of the root) in uninoculated plants. Most of the infection (67-71%) was caused by endophytes with fine hyphae such as those in the photographs (Fig. 30). Inoculation with G. mosseae L1 had no effect on the total amount of infected root but increased the level of infection caused by coarse endophytes from 10-15% to 30-42%. The total level of infection in roots of plants inoculated with G. caledonius tended to be less than in roots of uninoculated plants or plants inoculated with G. mosseae L1. The superphosphate treatment had little effect on infection.

The levels of root infection were less when sampled in 1979 (25-49%) than when sampled in 1978 (45-83%). This may have been related to the time of sampling as, in 1978, samples were taken in October but, in 1979, they were taken in December and much of the root cortex had sloughed away. However, the total levels of infection in clover roots at the end of 1979 were greater in treatments that were inoculated 20 months previously. The level of infection of clover treated with G. caledonius in 1978 was the least but was the most in 1979.

Table 46. The effect of Glomus mosseae (L1), G. caledonius and superphosphate on shoot dry weight and root infection of white clover grown at Sourhope on a brown earth (Experiment 15)

Cultivar	Super phosphate (kg P/ha)	Inoculant	1978 Yield (kg DM/ha)	1978 Infected Root (%)	1979 Yield (kg DM/ha)	1979 Infected Root (%)
Huia	0	None	344	83	1070	23
		G. mosseae L1	500	82	1271	46
		G. caledonius	216	45	598	46
	40	None	326	76	841	25
		G. mosseae L1	542	74	1126	33
		G. caledonius	200	64	1930	49
SED (15 df)			163	6.2	284	4.7

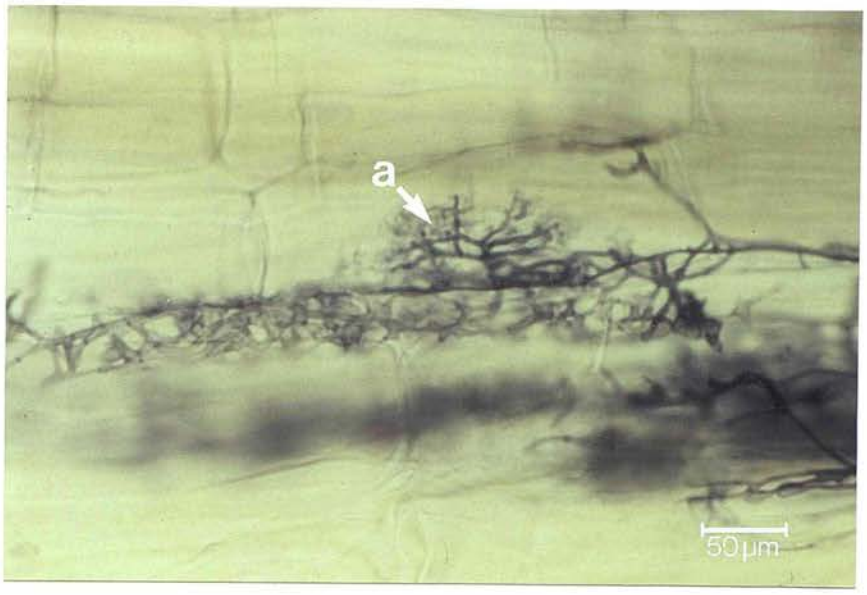


Figure 30. White clover roots infected with the fine endophyte, probably *Glomus tenuis* a) arbuscules; v) vesicles (see Hall, 1977a) (Experiment 15).
The photographs were taken by Dr. M.J.Daft, University of Dundee

Superphosphate reduced infection in roots of clover treated with L1, but not in roots infected by indigenous endophytes or in roots treated with G. caledonius.

Dry matter yield (Table 46)

In 1978 the yield from plants inoculated with L1 tended to be greater than the yield from uninoculated plants. The yield from plants inoculated with G. caledonius tended to be less than the yield from uninoculated plants but errors were high and none of the differences were significant. The superphosphate treatment had no effect on yield.

In 1979 the yields were similarly ordered except where the inoculant was G. caledonius with 40 kg P/ha. The yield from that treatment was significantly greater than any other treatment.

EXPERIMENT 16. The effect of four mycorrhizal inoculants and superphosphate on the growth of white clover on a brown earth at Cleish

EXPERIMENTAL

The effect of four mycorrhizal inoculants (G. macrocarpus, G. clarus and two isolates of G. mosseae) and superphosphate (0 and 50 kg P/ha) on the growth of white clover was measured in the field at Cleish on a second brown earth soil. The soil was ploughed and rotovated; 4 tonnes lime/ha, 100 kg K/ha, trace elements and none or 50 kg P/ha were applied. To ensure close contact between seed (sown at 5 kg/ha and inoculated

with rhizobia) and mycorrhizal inocula, the seed and inocula were placed in four drills which ran the length of each 2 x 4 m plot. Equal volumes (6 m^3/ha) of each inoculant or sterile sand were applied. The experiment was sown 21-22 June 1979 and harvested once on 11 October 1979. The site was photographed in August 1979 (Fig. 31).

RESULTS

Levels of root infection (Table 48)

Levels of root infection ranged between 53% and 79%. There were no significant differences between individual treatments; infection in uninoculated plants was similar to infection in inoculated plants, but overall the effect of superphosphate was to increase the level of infection.

Dry matter yield (Table 48)

Inoculation with G. mosseae (L1) reduced yield with superphosphate and tended to reduce yield in the absence of superphosphate. The other inoculants had no effect on yield. Superphosphate at 50 kg P/ha doubled the yield of clover.

The results from these experiments, which aimed to demonstrate the benefits of root infection with VA mycorrhizal fungi on the growth of white clover in hill pastures, together with the relevant information from the literature, will be discussed in the next section.

Table 48. The effect of four mycorrhizal inoculants and superphosphate on the dry matter yield and root infection of white clover grown at Cleish on a brown earth (Experiment 16)

Cultivar	Super-phosphate (kg P/ha)	Inoculant	Dry matter yield (kg/ha)	Root infection (%)
S184	0	None	39	70
		G. mosseae L1	21	53
		G. mosseae	37	57
		G. macrocarpus	48	73
		G. clarus	42	61
	50	None	98	65
		G. mosseae L1	57	77
		G. mosseae	94	64
		G. macrocarpus	100	79
		G. clarus	102	77
SED (27 df)			10.5	14.8

Figure 31. The site at Cleish photographed in August 1979. The largest of the three plots is the one described in experiment 16.

The discussion will relate to the success or otherwise of introduction of the inoculated endophytes, the effect of infection on plant growth and uptake of nutrients, and the effect on nodulation and nitrogen fixation.

DISCUSSION

The experimental work described in this part of the thesis has measured the effects of inoculation of white clover with several mycorrhizal endophytes, in three soils with different levels of applied phosphorus and under laboratory and field conditions. The work was aimed to assess the possible beneficial effects of inoculation of white clover for hill pasture improvement and not to investigate mechanisms of uptake of nutrients by mycorrhiza.

The three soils presented different problems for the establishment of a symbiosis which was beneficial to both the endophyte and the host. Experiments with the Lephinmore deep peat showed that the density of indigenous endophytes in this soil was low (<1 propagule/25 cm^3) and that in pot experiments (Experiments 8, 9 and 11) but not in the field (Experiment 14) inoculation with mycorrhizal fungi greatly increased plant growth. The lack of a growth response to inoculation in the field was at first sight surprising since inoculation had doubled the level of root infection and because one of the cultivars of white clover that was grown (S184) responded to applications of superphosphate as great as 200 kg P/ha. The reasons for the lack of response will be discussed later. The pot experiments (Experiments 8 and 9) also demonstrated that inoculation with mycorrhizal fungi resulted in more and larger root nodules, a greater rate of

nitrogen fixation and increased uptake of phosphorus, calcium and magnesium but not potassium when compared with uninoculated plants.

By contrast with the deep peat soil, which had a low density of indigenous endophytes, the two brown earth soils had a much greater density, one or more propagules of indigenous endophytes per cubic centimetre of soil. In both pot (Experiments 12 and 13) and field experiments (Experiments 15 and 16) uninoculated plants were highly mycorrhizal. However, white clover growth in the two brown earths differed in response to phosphorus. In the Cleish, but not in the Sourhope brown earth, plants responded to phosphorus and the results from the laboratory experiments agreed with those from the field. In laboratory experiments inoculation with mycorrhizal fungi had no effect on plant growth in either soil but at Cleish, in the year of sowing, inoculation with G. mosseae L1 decreased growth while at Sourhope in the second year G. caledonius increased growth when 40 kg P/ha was applied.

Many of the results can be interpreted from broad principles established by other workers but the tolerances of endophytes, hosts and, in the case of legumes, rhizobia, to environmental variables may differ. Since there have been few studies of the effect of environmental factors on VA mycorrhiza some of the interpretation of the results is speculative. The effects of inoculation are discussed under three main headings -

levels of infection, responses in growth, and effects on nodulation and nitrogen fixation.

LEVELS OF INFECTION

The measured levels of infection of inoculated plants grown in the three unsterile soils depended on several factors: the level of indigenous endophytes, the time the plants were sampled, the level of phosphorus in the soil or the plant, and the environmental conditions.

A plant root and a mycorrhizal propagule have to be fairly close before infection takes place (Powell, 1976b), therefore the greater the density of propagules in the soil, the more chance of infection and the faster the root is colonised. An inoculant added to a soil will increase the density of propagules and so increase the speed of infection (Daft and Nicolson, 1969b; Carling et al., 1979). In the early stages of development, infection by indigenous and introduced endophytes is probably additive (Abbott and Robson, 1978) although competition may occur in later stages when infections in the root cortex meet. Inoculants are generally placed in the rooting zone below the seed and have a greater chance of early infection and colonisation than indigenous endophytes which, in a well-mixed soil in a pot experiment or in a cultivated field soil, may be at a lower density but will be distributed throughout the soil. Therefore, it is to be expected that introduced endophytes will successfully colonise roots provided the inoculum is

strategically placed and the environmental conditions are favourable for infection. Where conditions are favourable, colonisation of the root will continue until most of the cortex is infected; the upper limit was as high as 95% of the cortex in Experiment 11 and only the root meristems and nodules were not infected. Therefore, if the density of indigenous inoculum is low, such as in the Lephinmore peat, it takes longer for the root cortex to be colonised than in soils with a greater density of indigenous inoculum such as the two brown earths used in the experiments. It is obvious that the measured level of infection is crucially affected by the time of sampling and this must be taken into account in the interpretation of results; in the experiments described here the measurements of infection were taken after most of the cortex was colonised.

In all the experiments in both field and laboratory the introduced endophytes successfully infected where it was possible to distinguish them from the indigenous endophytes. In the experiments at Lephinmore (deep peat) and at Sourhope (brown earth), the inocula and seed were broadcast and contact between the two was left to chance. At Lephinmore, the material was left on the surface and was liable to dry out although the wetness of the soil may have prevented this. At Sourhope, where the soil was cultivated, the seed and inocula were given some protection by raking them into the surface soil. Despite the random method of inoculation, the

introduced endophytes established themselves; the level of infection was doubled in the Lephinmore peat and in the Sourhope soil L1 partially replaced infection from indigenous fine endophytes. At Cleish (brown earth) the seed and inocula were drilled in the soil, and the drills were watered before they were covered with soil, and therefore the seed and inocula were damp and unlikely to dry out quickly; the developing root system was surrounded with inocula and germination and infection occurred in the dark. Unfortunately, in the Cleish soil the indigenous endophytes had coarse hyphae and could not easily be distinguished from the introduced ones, so it was not possible to tell whether the latter successfully infected the clover.

The problem arises as to what methods of inoculation are practical in the field. The methods used up till now have used relatively large quantities of infected soil or sand and, in the present experiments 4-6 m^3/ha of infected sand was used which would weigh 7-10 t/ha. Soil pellets, such as those described by Powell (1979) made in the proportion of 40:1, inoculum:seed, applied at a sowing rate of 3 kg white clover seed/ha (the rate recommended for seed mixtures for hill pastures in the UK) would result in about 0.12 t inoculant/ha. Such quantities of inoculant would be impossible to provide on a regular basis and would present problems in handling, particularly for hill land improvement. In many circumstances where soils are too wet to cultivate the

only way to sow improved pasture is to broadcast, and the inocula must be either broadcast or pelleted with the seed. It is suggested that agricultural research workers get together with chocolate manufacturers to produce a pellet with a light, moist, airy centre which dissolves in the soil but not in the spreader.

In some experiments, inoculation reduced the level of infection of white clover roots, i.e. inoculation with G. caledonius at Sourhope in the year of sowing and with G. mosseae L1 in the Cleish soil in the pot experiment. Therefore, there may be some competitive effects between endophytes at higher levels of infection.

It is of interest to note that Powell and Daniel (1978) found similar effects to those described above when white clover seedlings infected with G. tenuis were transplanted into six New Zealand hill soils in the greenhouse. Where indigenous infection was low (17%) inoculation increased infection; where indigenous infection was 50-64% of the root cortex, inoculation with G. tenuis replaced indigenous endophytes in some soils, but in others total infection was reduced after inoculation.

As a general rule, mycorrhizal infection is reduced as the level of phosphorus in the soil increases (Daft and Nicolson, 1969a) but the tolerances of species and strains may differ and may be related to the phosphorus level of the soil from which the fungus was isolated. However, Porter et al. (1978) did not find that two

inocula collected from soils with very different histories of superphosphate application over a period of 10 years differed in their sensitivity to phosphorus during colonisation. Sparling and Tinker (1978c) found that the fine endophyte and a coarse endophyte from the same soil had different tolerances of the same level of phosphate.

In the pot experiments the effect of phosphorus on infection was not very great. Levels of infection by indigenous endophytes in the Lephinmore peat (Experiment 8) and the Sourhope brown earth (Experiment 12) were reduced by phosphorus, as were the levels of infection in plants which had been inoculated with G. macrocarpus and G. clarus in the Cleish brown earth (Experiment 13) but the level of infection with L1 in all soils was unaffected. However, in the field, with higher levels of phosphorus than those applied in pots, the level of infection in Huia treated with L1 was reduced (Experiments 14 and 15) whereas, by contrast, application of phosphorus to the brown earth at Cleish increased the level of infection with all introduced endophytes (Experiment 16). The latter observation for the Cleish soil, which is extremely low in available phosphorus, agrees with the results of Abbott and Robson (1977b) who attributed the effect to greater root growth. It is of interest to note that, although not significant, infection with indigenous endophytes in the uninoculated treatment at Cleish was slightly decreased by added

phosphorus.

If the effect of phosphorus was a direct effect of concentration in the soil solution, there should be a general reduction in level of infection as phosphorus is applied to a particular soil but, if the effect is indirect through level of phosphorus in the plant tissues, as most of the information suggests (see Introduction, p.187), other considerations, such as rate of growth of the plant, would interact. For example, with a moderate to low dressing of phosphorus, plants deficient in potassium may accumulate phosphorus in the tissues (Part I, Experiment 1) and so may reduce the level of infection. The experiments described here do not help to answer this question but they do show that applications of phosphorus to the soil were not the major factor which reduced infection.

Among other factors which may be important for establishment of infection in improved hill pastures are temperature, waterlogging and soil pH. Since VA mycorrhizas have been recorded in plants in most climatic regions of the world (Hayman, 1978) there is no reason to suppose that temperature may restrict development, but endophytes do have different tolerances (Schenck and Schroder, 1974; Schenck *et al.*, 1975) and they seem to be related to the region in which the strain was isolated. Daft *et al.* (1980) have recorded mycorrhizal development in bluebells below 5^{0}C with *Acaulospora laevis* and *G. caledonius*. The strain most used in the work

described in this thesis was G. mosseae L1 which was isolated from a Libyan soil, and although a first appraisal might suggest that it was unlikely to be suitable for Scottish conditions, after a winter at Lephinmore, the level of infection of the roots of plants from inoculated plots with this endophyte was double the infection of plants from uninoculated plots (Table 45). At Lephinmore, while infection would develop slowly because of the low density of inoculum the factor which probably restricted the absolute level of infection most in the field experiments was waterlogging. Mycorrhizal plants are not generally found in waterlogged soils (Mejstrik, 1965) and, therefore, at Lephinmore, where the soil is waterlogged for most of the year, infection levels were always lower than at Sourhope and Cleish on soils which are rarely waterlogged. While other factors may be involved at Lephinmore, it is suggested that waterlogging may be the major one. Level of infection may vary as the wetness of the soil varies, with the greatest level of infection in the summer when the plants are growing rapidly under relatively dry conditions. Khan (1974) found that mycorrhizal plants of Ipomoea carnea growing on drier soils became non-mycorrhizal when temporarily waterlogged during the monsoon and suggested that wet growing conditions resulted in the absence of mycorrhizas. It is noteworthy that, in the pot experiments (Experiments 8 and 11), using partially dried shredded deep peat, levels

of root colonisation after inoculation reached 95%.

Soil pH also affects the establishment of the infection. However, to grow white clover successfully, soils have to be limed to pH 5.0-6.0 (see Part I, p. 17) and infection always occurred in the present experiments which were limed to this level. It is unlikely, therefore, that low pH per se would inhibit infection, but Mosse (1972) has presented some evidence that endophytes have different preferences for soil pH, and Skipper and Smith (1979) showed with soybeans that specific cultivar-fungal response was dependent on soil pH. Further investigation is needed to clarify the effect of soil pH and liming on the colonisation of white clover by different fungal endophytes in hill soils.

Therefore, it is confirmed that, provided the inoculant is strategically placed and is tolerant of the conditions imposed in the field, it can be successfully introduced on to white clover roots in a hill pasture.

The next and probably the most important question for use of mycorrhiza in hill and upland agriculture is "Can inoculation increase growth?"

GROWTH RESPONSES TO INOCULATION

The work reported here is mainly concerned with the beneficial effects of VA mycorrhizas on phosphorus uptake in white clover which leads to growth responses when clover is grown in hill soils which are low in available phosphorus. Uptake of other elements has been

implicated and the relevant points will be discussed at the end of this section.

Mycorrhizal plants are thought to obtain phosphorus from the same source as non-mycorrhizal plants by effectively increasing the absorptive area of the root (see Introduction, p.170). Pairunan et al. (1980) have recently examined several reports which claim that mycorrhizal plants can absorb phosphorus from sources unavailable to non-mycorrhizal plants and, from their own experiments, have found that responses to mycorrhizas were not observed when phosphorus was not available for uptake by the plant or when phosphorus supply did not limit plant growth. Their results agree with this author's. In the Lephinmore peat, in Experiment 9, where indigenous infection was low and the uninoculated plants were essentially non-mycorrhizal, the plants responded to inoculation with mycorrhizas at 40 kg P/ha early in their growth but, where 160 kg P/ha was applied, the response developed in later harvests, probably when the added phosphorus became less available (Barrow and Shaw, 1975). It is interesting to note that the inoculant seemed to be effective at increasing growth after a period when plant growth was not limited by phosphorus. This leads one to believe that inoculation, when the seed is sown, may be most beneficial in later growth of the pasture, provided the level of other nutrients essential for growth are maintained and when available phosphorus in the soil, either from fertilizers

or from mineralisation after liming, has declined.

Despite large increases in growth after inoculation of white clover in the deep peat in the pot experiments, there were no responses to inoculation in the field, even though inoculated plants had twice the level of infected root than uninoculated plants. Uptake of phosphorus by the fungi may have a different tolerance to environmental factors than infection; the low temperature on the hills or the wetness of the soil may have limited P-uptake and transfer by the Libyan strain of G. mosseae. Uptake and translocation of phosphorus by fungal hyphae, which is probably an active metabolic process (Cooper and Tinker, 1978; Cress et al., 1979), may be restricted in waterlogged soils which are poorly aerated. In the absence of information for mycorrhizal fungi, analogies may be drawn with the effect of oxygen tension on translocation of phosphate in barley (Loughman, 1969).

It was interesting to note that S184 responded more to phosphorus than did Huia in the Lephinmore peat when compared both in a pot experiment when gafsa was applied (Experiment 11) and in the field experiment where a very high level of superphosphate was applied (Experiment 14). From the available information, no explanation can be given for the different response of the two cultivars to added phosphorus except to say that ecotypes of white clover are known to respond differently to phosphorus (Snaydon and Bradshaw, 1962b). Where tricalcium phosphate was applied in the pot experiments (Part I,

Experiment 1 and Part II, Experiment 8), Huia responded to the element. The results emphasise the danger of assuming that all cultivars of white clover behave identically and indicate that caution is needed when extrapolating conclusions obtained with one particular cultivar, to white clover in general.

Results from Sourhope bring up the question of whether different endophytes have different abilities to promote growth or whether the effects on growth are related to the amount of root infection during the growth of the plant. Data from the first harvest in the Sourhope field trial (Experiment 15) illustrate the point, since there were large differences in growth between inoculation treatments, although these failed to reach statistical significance because of the large error. However, the level of infection and growth of plants inoculated with G. caledonius tended to be less than the control so the results with this endophyte can be explained solely by differences in infection. However, the growth of plants inoculated with G. mosseae L1 tended to be greater than the control but levels of infection were the same. Therefore, it is interesting to speculate either that L1 was more able to increase growth than indigenous strains or that inoculation resulted in more infection early in the season with a consequent increase in growth.

Where there was significant response to mycorrhizal inoculation at Sourhope in the second year with

G. caledonius given 40 kg P/ha, the data suggest that the total level of infection is more important than the relative efficiency of endophytes because the level of infection by G. caledonius was greater than for the control and L1 (Experiment 15). However, the samples of root for assessment of infection were taken in December when the root cortex was decaying and may not have been the same as levels of infection during the growth period. The low yield of plants inoculated with G. caledonius in the absence of phosphorus in the second year is difficult to explain, particularly when the level of infection was relatively high. The endophyte was isolated from a fertile soil which had been part of a market garden for severel years and it may be that this endophyte is not efficient at phosphorus uptake in soils low in phosphorus but is able to infect and so is a drain on the photosynthate of plants.

Responses by white clover to inoculation in unsterilised soil in the field have been obtained by Powell (1977band 1979) in New Zealand, and by Hayman and Mosse (1979) in Wales. The work by Hayman and Mosse (1979) and some of the work by Powell (1977b) used seedlings pre-inoculated in the laboratory and transplanted into the field. Transplants are open to the criticism that, when transplanting, the seedlings are not equal in all but mycorrhizal infection and, in addition, it is difficult to see any use for them in farm practice. It is of interest to note that, even

with transplants, the benefits of inoculation were more evident in the second than in the first year (Hayman and Mosse, 1979), which agrees with this author's findings when white clover seed was inoculated with G. caledonius at Sourhope (Experiment 15). Powell (1977b) sowed white clover above pads of indigenous inoculum and E3 in the field and found that E3 promoted more growth than indigenous species but, when he used transplants infected with E3, they did not promote growth as well as those infected with indigenous fungi. In a later experiment, clover seed pelleted with G. tenuis or E3 with Gigaspora margarita, grew better than clover seed pelleted with indigenous fungi when arranged on a clipped pasture in the field. This latter experiment is the nearest to agricultural practice but, as discussed earlier, even the production of the amount of inoculum for the pellets would present problems and the distribution of the pellets in the field would present others.

The Cleish soil presented a different problem in that the soil contained a relatively high density of inoculum with moderate infection in the field and the infected plants responded to phosphorus unlike the Sourhope soil where there was no response to phosphorus. Since the moderate levels of infection in the laboratory and field were not caused by waterlogging, there may have been some factor in the soil to which the endophytes, particularly L1, were sensitive. In the early stages of growth of the clover in the field, the plants

infected with G. clarus looked bigger than the controls and this may have been a transient effect caused by quicker infection during early growth. Unfortunately, the growth depression caused by L1 in the field experiment (Experiment 16) was not associated with a lower level of root infection, and the lower level of root infection in the laboratory (Experiment 13) was not associated with a reduction in dry weight. However, it is tempting to conclude that slower colonisation of the root by L1 at Cleish may explain the depression in growth, but other explanations must be sought.

There is a great deal of speculation about the nature of depressions in plant growth caused by mycorrhizal fungi, many of which are transitory (see Introduction, p.172). Possibilities are that mycorrhizas absorb so much phosphorus that the element becomes toxic in the plant tissues (Mosse, 1973b), that plant roots and fungal hyphae compete for phosphorus in the soil early in growth (Cooper, 1975), or for carbohydrates in the plant (Hall, 1977). Competition for other elements and interactions involving trace element and production by the fungus of chemicals toxic to the plant might also be involved. The explanation of Mosse (1973b), while possible in some situations, is not applicable in many cases since depressions often occur with relatively low levels of soil phosphorus (Cooper, 1975; Hall et al., 1977; Experiment 16). Of the other explanations, competition for nutrients (phosphorus or trace elements) by

mycorrhizal fungi are possible since in these experiments depressions occurred when there was a fairly dense number of plants in the pots (Experiment 9) or in the rows in the field (Experiment 16). However, these hypotheses do not explain why one endophyte depressed growth and another did not: the most probable explanation would appear to involve the carbohydrate balance in the plant.

VA mycorrhizas are generally thought of as mutualistic symbioses where the plant provides photosynthate to the fungus and the fungus supplies a nutrient, principally phosphorus, to the plant. Only when the supply of a nutrient from the soil limits plant growth is there a growth response in the plant to mycorrhiza. Any environmental factor which upsets the uptake and transfer of phosphorus to the plant by the fungus may not stop transfer of carbohydrate from the host to the fungus. Therefore, the endophyte would drain the host of photosynthate and deficiency of phosphorus would reduce production of photosynthate, the result being a depression in growth. However, Bevege et al. (1975) found that VA fungi were not large sinks for photosynthate in subterranean clover but Abbott and Robson (1977b) and Pairunan et al. (1980) have found that, for a given concentration of phosphorus in tops, non-mycorrhizal plants produced more dry weight of tops than did mycorrhizal plants when provided with superphosphate. For the same weight of tops, the concentration of phosphorus for mycorrhizal plants was up to twice that

for non-mycorrhizal plants, which suggests that the fungus may take a considerable amount of the carbohydrate from the plant. Any environmental factors which adversely affect phosphorus uptake may vary in duration and this would explain why some growth depressions are transitory and others last longer. Until more is known about the effect of environmental variables on the symbiosis, the views presented here are only speculative.

Tinker (1975a) suggested that uptake of nutrients which are supplied to the root by mass flow is unlikely to be enhanced by mycorrhizal infection. Barber et al. (1962) demonstrated from data for 147 soils in the USA that supply of calcium and magnesium to maize was sufficient from mass flow, but that only one-tenth of the potassium and one-eightieth of the phosphorus was so supplied. Therefore, it might be expected that uptake of potassium as well as phosphorus could be enhanced by mycorrhizas. However, the rate at which potassium is diffused in the soil is much quicker than phosphorus (Nye and Tinker, 1977) and this may account partly for the apparent lack of effect of mycorrhiza on the uptake of potassium in Experiments 8 and 9. There is conflicting evidence in the literature for the effect of mycorrhizas on the uptake of potassium. Some authors found mycorrhiza lowered the concentration of potassium (Gerdemann, 1964; Holevas, 1966; Deal et al., 1972; Kleinschmidt and Gerdemann, 1972) and this agrees with the data in Experiment 8 (Table 37). If this effect of

mycorrhiza on potassium concentration is due to enhanced uptake of phosphorus, the data in Experiment 1 (Part I, Tables 8 and 9) also support this finding. Other workers found either an increase in concentration of potassium in mycorrhizal plants (Mosse, 1957; Baylis, 1959) or no change in concentration (Ross and Harper, 1970; Powell, 1975b). All found that uptake of potassium increased because of a corresponding increase in growth of mycorrhizal plants. In Experiment 8 (Table 37) there was no increase in uptake of potassium but this may have been because the plants were near the deficiency level (see Experiment 1, Table 9). There is, however, another explanation of the lack of effect of mycorrhiza on uptake of potassium which arises from work with sheathing mycorrhizas. The latter are known to produce hormones and cytokinins which increase the permeability of cell membranes and increase the passive efflux of potassium (Bowen and Theodorou, 1973). It has also been observed that, at low oxygen concentrations, such as can occur in waterlogged soils, potassium is more rapidly released from roots infected with sheathing mycorrhizas (Harley, 1969). Similar mechanisms may occur with VA mycorrhizas.

In Experiment 9 the effect of mycorrhiza on growth was not conclusively an effect of enhanced phosphorus nutrition. Of the nutrients measured, none was below the critical concentration in the non-mycorrhizal plants with low phosphorus (see Part I, Experiment 1). Inoculation resulted in a lowering of concentration of all the

elements. It is possible, therefore, that the enhanced growth was caused by uptake of another element, such as zinc and sulphur (see Introduction, p. 169), which were not measured in the present experiments. The fact that the plants responded to the higher level of phosphorus does, however, suggest that the growth response due to mycorrhiza was primarily caused by a greater uptake of phosphorus.

NODULATION AND NITROGEN FIXATION

The results of Experiments 8 and 9 agree with those of other workers who found inoculation of legumes with mycorrhizal fungi in sand and soil low in phosphorus increased nodule numbers and nitrogen fixation (measured by acetylene reduction) (Crush, 1974; Daft and El Giahmi, 1974, 1975; Mosse et al., 1976; Smith and Daft, 1977; and others). With the low level of phosphorus in Experiment 9, the number of nodules was increased only indirectly through increased root growth (i.e. the number of nodules per gram root was the same for inoculated and uninoculated plants). The important thing was that the proportion of large nodules increased from 1 in 15 in uninoculated plants to 1 in 3 in inoculated plants (Table 38). It was very noticeable that the latter proportion was similar to the proportion of large nodules on plants given an adequate amount of phosphorus. In subterranean clover, Smith et al. (1979) found that in mycorrhizal and non-mycorrhizal plants of about the

same size, nodule numbers differed little but the nodule volume was greater in mycorrhizal plants.

In Experiment 9, the total number of nodules on plants given the high level of phosphorus was greater than on mycorrhizal plants given low phosphorus. Other workers (Crush, 1974; Abbott and Robson, 1977b)have generally found that inoculation and phosphorus have similar effects on nodulation. Since mycorrhiza will only increase the phosphorus concentration in the plant, the results may indicate that the level of phosphorus in the soil is important for infection by rhizobia.

Data from Experiment 8 show that, although the dry matter produced by mycorrhizal plants given 40 kg P was about the same as non-mycorrhizal plants given 160 kg P, the rate of nitrogen fixation (measured by acetylene reduction) was significantly greater in the latter plants. Moreover, the amount of nitrogen in the shoots of these plants tended to be greater than for those which were mycorrhizal and given 40 kg P, but this just failed to reach statistical significance (Table 38). Observations of a similar nature were made in Experiment 9.

These results suggest that mycorrhizal fungi were unable to enhance growth and nitrogen fixation in parallel and to the same degree as a high level of added phosphorus. This view contrasts somewhat with that of Smith et al. (1979) who suggested that effects of mycorrhizal fungi on nodulation and nitrogen fixation

early in the growth of a legume (subterranean clover) precede those on dry matter production. Further experimentation aimed specifically to resolve these uncertainties is required.

FUTURE WORK

The present experiments, though producing variable, and sometimes not completely explicable, responses by white clover to inoculation with mycorrhizal fungi in hill soils, have shown that benefits to establishment and growth can occur. The results are judged sufficiently promising to justify further work in the future, the nature of which is briefly outlined below with emphasis on what is required for white clover in hill pastures of the United Kingdom.

At the moment, agriculturists, in their attempt to exploit the VA mycorrhizal association, are working with fragmentary knowledge of how mycorrhizal fungi react to environmental variables, what the possible range of variability is, and whether the growth stimulation is primarily caused by differences in rate of uptake by a unit amount of hyphae, in the absolute amount of infection or in the rate of development of the infection.

Thus, there is a need to study the annual cycle of development and colonisation of mycorrhiza in pastures so that the weaknesses in the indigenous cycle can be identified and remedied. There is also a need to screen endophytes from existing hill pasture in comparison to

standard endophytes, e.g. Glomus fasciulatus (E3) and endophytes from other pasture sites for tolerance of existing, and adaptability to changing, environmental conditions. It may be possible to isolate endophytes which infect and take up phosphate under conditions of low temperature (see Daft et al., 1980) and in wet soil (see p.197).

Since there is mounting evidence of interactions between cultivars of legume and strain of Rhizobium (Mytton, 1975; Hardason and Jones, 1979a) and of wide variability between Rhizobium in tolerance of low temperatures (Vernon, 1978; Hardason and Jones, 1979b), and of differential tolerance to temperature by a sub-terranean clover/Rhizobium/mycorrhizal combination (Smith and Bowen, 1979), great importance is attached to the study of interactions between the three organisms and their environment. Mycorrhizal endophytes suitable for establishment on hill land should be collected from established white clover plants with full details of site and soil type, including pH and phosphorus and nitrogen status, for screening against a range of cultivars of white clover and strains of Rhizobium, in the laboratory under conditions which resemble those in the field. The most promising combinations should then be tested in the field.

As yet, there are no satisfactory methods of identifying different species of fungi (see Abbott and

Robson, 1979, for some of the problems), let alone different strains of a species. Abbott and Robson (1978) have found contrasting effects between strains of apparently the same species of endophyte. Methods other than the classical method based on spore morphology are required. It may be necessary to have a separate functional agricultural classification rather than the traditional one, and a scheme confined to clover with reference collections based at one centre. Work in this direction would, of course, be greatly stimulated by ability to grow endomycorrhizal fungi on culture media without a host, particularly since it seems that non-sporulating types may be of most use in pastures.

Another important field of investigation is to differentiate the effects of environment on infection, colonisation, uptake and transfer of nutrients by mycorrhizal fungi. For example, it is no use having an endophyte which colonises well under conditions adverse for nutrient uptake, particularly of phosphorus, or to have an endophyte efficient at transferring phosphorus but which is unable to infect and colonise in sufficient amount when conditions are cold and wet. Further clarification is needed of the relative importance of level of soil phosphorus and concentration of phosphorus in plant roots on infection and colonisation of white clover by mycorrhizal fungi. The existence of specific cultivar-fungal responses to pH and to

liming needs to be investigated in white clover on hill soils.

The effects of mycorrhiza on the uptake of nutrients other than phosphorus by white clover in hill soils requires further work. This applies particularly to trace elements which may affect the growth and functioning of nodules, e.g. molybdenum,, and those which do not affect plant growth but are of great importance to animals grazing the plants, e.g. copper. Because of the importance of potassium to the successful growth of white clover in peaty hill soils (Part I), it is especially important to clarify the role of mycorrhizal fungi in potassium uptake, transfer and efflux.

There is a need to study the energetics of the symbiosis in much more detail and other possible mechanisms of growth depression by mycorrhiza so that they can be avoided, or at least minimised in agricultural practice.

The relationships between earliness and magnitude of infection, nodulation and nitrogen fixation, plant growth, photosynthesis and partitioning of metabolites, also require examination.

A key area for practical study is to develop methods of inoculation, particularly for uncultivatable soils such as deep peats, which are economically and logistically feasible for the producer of inoculum, the seed merchant and the farmer. Methods must allow nearness of root and inoculum and protect inoculum from adverse

conditions such as dessication. Probably the most promising method developed as yet is seed pelleting, but the materials used to date are generally too heavy for routine practical use. New materials to carry and protect seed and inoculum must be developed, and the use of pellets with inocula sown separately from the seed also merit investigation.

SUMMARY

The present investigations were undertaken to study the responses in dry matter production and nutrient content of white clover growing in hill soils to additions of the major nutrients (Part I) and the benefits, or otherwise, of mycorrhiza to the establishment, growth and nutrient content of white clover (Part II).

The work with mycorrhizas described in Part II arose from observations made during the initial experiment in Part I (see p.212). Studies on this subject were expanded because of interest in the phosphate nutrition of white clover. Results from the two lines of study complement each other and, taken together, they provide a clear and unified picture of the nutrient requirements for growth of this plant in fertilized hill soils. The only observations which were unexpected in this study were the lack of response by the cultivars of white clover Huia to phosphorus when applied as gafsa phosphate in the laboratory (Experiment 11) or as superphosphate at a very high level (200 kg P/ha) under field conditions (Experiment 14). By contrast, the cultivar S184 responded to these treatments and this difference has been discussed on p.260. However, it is noteworthy that where tricalcium phosphate was the form of fertilizer applied to the deep peat soil, Huia responded in similar manner (Experiment 1, Part I, and Experiments 8 and 9, Part II). Four soils representing three of the four main groups of hill soil were used, although it was not

possible to carry out all experiments or analyses with each soil. Experiments were carried out both in the field and in pots containing soils from the field placed either in a glasshouse or a growth room. The detailed results are discussed in each part of the thesis and only the key points are presented here.

PART I

In laboratory experiments, white clover inoculated with Rhizobium was not found to respond to nitrogen nor did this element interact with phosphorus or potassium. However, the plants responded markedly to phosphorus and potassium; the response to one element was greatly influenced by the level of application of the other. There was a positive response to lime up to a pH of 5.5; thereafter, further lime reduced growth, apparently through its effect on the phosphorus nutrition of the plant. There was little response to magnesium. Field experiments with an established pasture on deep peat soil confirmed that lack of phosphorus and potassium can severely limit the growth of white clover.

It was made clear in the discussion (pp.138-145) that it was not possible to give fertilizer recommendations from the pot experiments which had not been tested against field trials. However, the results emphasise the need for balanced applications of fertilizers, particularly for phosphorus and potassium, on deep peat. The amount of potassium applied should be equal to or more than

that of phosphorus and it is suggested that significant responses would follow application of 60 kg P/ha with at least 80 kg K/ha. The results also stress the need for applications of soluble phosphorus fertilizer when soils are limed.

Critical concentrations of phosphorus, potassium and magnesium in the dry matter of the shoot were found to be 0.20% P, 0.9% K and 0.29% Mg. Although the critical concentration of calcium was not determined precisely the data suggests that a concentration in whole shoots of 1% or less restricts growth. The use of shoot analysis and critical concentrations to determine the need for maintenance dressings of fertilizer is suggested to complement soil analysis and assessments of the botanical composition of improved hill pastures.

PART II

Mycorrhizal fungi were successfully introduced into the roots of white clover in both laboratory and field experiments; the responses to inoculation depended on soil type, introduced endophyte, the presence of indigenous endophytes and the environmental conditions. In laboratory experiments with a deep peat soil there were large responses in dry matter production and nutrient uptake, coupled with beneficial effects on nodulation and nitrogen fixation. Low temperatures and the wetness of this soil were probably the major environmental factors which prevented growth responses in the field.

With two brown earth soils in the laboratory there were no responses in dry matter production, possibly because the soils contained a high density of indigenous endophytes. However, Glomus caledonius did significantly increase yield in the field on one of the soils during the second year of growth. By contrast, on the other brown earth soil, Glomus mosseae (L1) significantly depressed yield in the year of sowing.

It was concluded that mycorrhizas should be collected from well-established white clover pastures throughout Britain and screened in the laboratory, care being taken to match the mycorrhizal endophytes with both cultivars of white clover and strain of Rhizobium.

The results of this study have laid the basis for further nutrient work with other hill soil types and with mixed swards of white clover and companion grasses in the field. It has also shown that, although mycorrhizas have varying effects on clover growth in the field, some can be very beneficial and further investigation is justified.

REFERENCES

ABBOTT, L.K. and ROBSON, A.D. (1977a). The distribution and abundance of vesicular-arbuscular endophytes in some Western Australian soils. Aust. J. Bot., 25, 1-8.

ABBOTT, L.K. and ROBSON, A.D. (1977b). Growth stimulation of subterranean clover with vesicular-arbuscular mycorrhiza. Aust. J. agric. Res., 28, 639-649.

ABBOTT, L.K. and ROBSON, A.D. (1978). Growth of subterranean clover in relation to the formation of endomycorrhizas by introduced and indigenous fungi in a field soil. New Phytol., 81. 575-585.

ABBOTT, L.K. and ROBSON, A.D. (1979). A quantitative study of the spores and anatomy of mycorrhizas formed by a species of Glomus, with reference to its taxonomy. Aust. J. Bot., 27, 363-375.

ADAMS, A.F.R. and LOWTHER, W.L. (1970). Lime, inoculation and seed coating in the establishment of oversown clovers. N.Z. Jl. agric. Res., 13, 242-251.

ADAS (1976). Grassland practice No. 5. Phosphate on grassland. Short term leaflet 101, HMSO London.

ADAS (1978). Grassland practice No. 4. Potash on grassland. Short term leaflet 90. HMSO London.

ADAS (1978). Redesdale Annual Review 1978. HMSO London, 40-47.

ADAS (1979). Fertilizer recommendations for agricultural and horticultural crops. GF1. HMSO London.

ALLAWAY, W.H. (1975). The effect of soils and fertilizers on human and animal nutrition. USDA Agric. Inf. Bull. 378.

ALLEN, S.E., Ed. (1974). Chemical analysis of ecological materials. Oxford: Blackwell Scientific Publications.

ANDREW, C.S. (1960). The effect of phosphorus, potassium and calcium on the growth, chemical composition and symtoms of deficiency of white clover in a subtropical environment. Aust. J. agric. Res., 11, 149-161.

ANDREW, C.S. (1976). Effect of calcium, pH and nitrogen on the growth and chemical composition of some tropical and temperate legumes. I. Nodulation and growth. Aust. J. agric. Res., 27, 611-623.

ANDREW, C.S. (1977). Nutritional restraints on legume-symbiosis. In: Vincent, J.M., Whitney, A.S. and Bose, J. (eds). Exploiting the legume-Rhizobium symbiosis in tropical agriculture. Misc.Publ. Coll. Trop. Agric. Univ. Hawaii, 145, 253-274.

ANDREW, C.S. and JOHANSEN, C. (1978). Differences between pasture species in their requirements for nitrogen and phosphorus. In: Wilson, J.R. (ed) Plant relations in pastures. Proc. Symp. Brisbane, 1976. CSIRO, 111-127.

ANDREW, C.S. and NORRIS, D.O. (1961). Comparative responses to calcium of five tropical and four temperate pasture legume species. Aust. J. agric. Res., 12, 40-55.

ANSARI, A.Q. and BOWLING, D.J.F. (1972). Measurement of the transroot electrical potential of plants grown in soil. New Phytol., 71, 111-117.

ARMSTRONG, R.H. and EADIE, J. (1973). Lamb growth on grass and clover diets. Proc. Br. Soc. Anim. Prod. 2, 68.

ASAI, T (1934). Uber das Vorkommen und die Bedeutung der Wurzelpilze in den Landplazen. Jap. J. Bot. 7, 107-150.

ASAI, T (1944). Uber die Mykorrhizenbildung der Leguminosen Pflanzen. Jap. J. Bot., 13, 463-485.

ATKINSON, D. (1973). Some general effects of phosphorus deficiency on growth and development. New Phytol., 72, 101-111.

AZCON, R., AZCON-G de AGUILAR, C. and BAREA, J.M. (1978a). Effects of plant hormones present in bacterial cultures on the formation and responses to VA mycorrhiza. New Phytol., 80, 359-364.

AZCON, R., MARIN, A.D. and BAREA, J.M. (1978b). Comparative role of phosphate in soil or inside the host on the formation and effects of endomycorrhiza. Pl. Soil, 49, 561-567.

BAGYARAJ, D.J. and MENGE, J.A. (1978). Interaction between VA mycorrhiza and their effects on rhizosphere microflora and plant growth. New Phytol., 80, 567-573.

BAKER, D.A. and HALL, J.L. (eds)(1975). Ion transport in plant cells and tissues. Amsterdam. North Holland Publishing Co.

BALTRUSCHAT, H., SIKORA, R.A. and SCHONBECK, F. (1973). Effect of VA mycorrhiza Endogone mosseae on the establishment of Thielaviopsis basicola and Meloidogyne incognita on tobacco. 2nd Int. Cong. Pl. Pathol., 661 (Abstr.).

BARBER, S.A., WALKER, J.M. and VASEY, E.H. (1962). Principles of ion movement through the soil to the plant root. Trans. Int. Soil Conf. New Zealand. Commissions IV and V 121-124.

BAREA, J.M., AZCON, R. and HAYMAN, D.S. (1975). Possible synergistic interaction between Endogone and phosphate-solubilizing bacteria in low phosphate soils. In: Sanders, F.E., Mosse, B. and Tinker, P.B. (eds) Endomycorrhizas. London : Academic Press, 409-417.

BARROW, N.J., MALAJCZUK, N. and SHAW, T.C. (1977). A direct test of the ability of vesicular-arbuscular mycorrhiza to help plants take up fixed soil phosphate. New Phytol. 78, 269-276.

BARROW, N.J., and SHAW, T.C. (1975). The slow reactions between soil and anions. 2. Effect of time and temperature on the decrease in phosphate concentration in the soil solution. Soil Sci. 119, 167-177.

BAYLIS, G.T.S. (1959). Effect of vesicular-arbuscular mycorrhizas on growth of Griselina littoralis (Cornaceae). New Phytol 58, 274-280.

BAYLIS, G.T.S. (1967). Experiments on the ecological significance of phycomycetous mycorrhizas. New Phytol. 66, 231-243.

BAYLIS, G.T.S. (1969). Host treatment and spore production by Endogone. N.Z. Jl. Bot. 7, 173-174.

BAYLIS, G.T.S. (1970). Root hairs and phycomycetous mycorrhizas in phosphorus-deficient soil. Pl. Soil, 33, 713-716.

BAYLIS, G.T.S. (1972a). Minimum levels of available phosphorus for non-mycorrhizal plants. Pl. Soil, 36, 233-234.

BAYLIS, G.T.S. (1972b). Fungi, phosphorus, and the evolution of root systems. Search 3, 257-259.

BENNETT, D. and BOWDEN, J.W. (1976). "Decide" - an aid to efficient use of phosphorus. In: Blair, G.J. Prospects for improving the efficiency of phosphorus utilization. Reviews in Rural Science III, Univ. New England, 77-81.

BEVEGE, D.I. and BOWEN, G.D. (1975). Endogone strain and host plant differences in development of vesicular-arbuscular mycorrhizas. In: Sanders, F.E., Mosse, B. and Tinker, P.B. (eds) Endomycorrhizas. London : Academic Press, 77-86.

BEVEGE, D.I., BOWEN, G.D. and SKINNER, M.F. (1975). Comparative carbohydrate physiology of ecto- and endomycorrhizas. In: Sanders, F.E., Mosse, B. and Tinker, P.B. (eds) Endomycorrhizas. London : Academic Press, 149-174.

BIELESKI, R.L. (1973). Phosphate pools, phosphate transport and phosphate availability. A Rev. Pl. Physiol. 24, 225-252.

BIRSE, E.L. (1968). Hill-land vegetation in Scotland. BGS Occ Symp. 4, 16-19.

BLACK, R.L.B. and Tinker, P.B. (1977). Interaction between effects of vesicular-arbuscular mycorrhiza and fertiliser phosphorus on yields of potatoes in the field. Nature, Lond. 267, 510-511.

BOUMA, D. (1967). Growth changes of subterranean clover during recovery from phosphorus and sulphur stresses. Aust. J. biol. Sci. 20, 51-66.

BOUMA, D. and DOWLING, E.J. (1966). The physiological assessment of the nutrient status of plants. II. The effect of the nutrient status of the plant with respect to phosphorus, sulphur, potassium, calcium or boron on the pattern of leaf area response following transfer to different nutrient solutions. Aust. J. agric. Res. 17, 633-646.

BOUMA, D. and DOWLING, E.J. (1976). The relationship between the phosphorus status of subterranean clover plants and the dry weight responses of detached leaves in solutions with and without phosphorus. Aust. J. agric. Res. 27, 53-62.

BOUMA, D., DOWLING, E.J. and WAHJOEDI, H. (1979). Some effects of potassium and magnesium on the growth of subterranean clover (Trifolium subterranean). Ann. Bot. 43, 529-538.

BOWEN, G.D. and THEODOROU, C. (1973). Fungal growth around seeds and roots. In: Marks, G.C. and Kozlowski, T.T. (eds) Ectomycorrhizae. New York : Academic Press, Chapter 4.

BOWEN, G.D. (1973). Mineral nutrition of ectomycorrhizae. In: Marks, G.C. and Kozlowski, T.T. (eds) Ectomycorrhizae. New York : Academic Press, Chapter 5.

BOWLING, D.J.F. (1976). Uptake of ions by plant roots. London : Chapman and Hall.

BROADBENT, F.E. and CLARK, F. (1965). Denitrification. In: Bartholomew, W.V. and Clark, F.E. (eds). Soil nitrogen. Agronomy No. 10. Am. Soc. Agron.

BROWN, B.A. (1957). Potassium fertilization of Ladino clover. Agron. J. 49, 477-480.

BUCKMAN, H.O. and BRADY, N.C. (1969). The nature and properties of soils, 7th ed. London : The MacMillan Co. Collier-MacMillan Ltd., 494-495.

BURPEE, L.L. and COLE, H. (1978). The influence of alachlor, trifluralin and diazinon on the development of endogenous mycorrhizae in soybeans. Bull. Env. Contam. Toxic 19, 191-197.

CALLOW, J.A., CAPACCIO, L.C.M., PARISH, G. and TINKER, P.B. (1978). Detection and estimation of polyphosphate in vesicular-arbusculur mycorrhizas. New Phytol. 80, 125-134.

CARADUS, J.R. and EVANS, P.S. (1977). Seasonal root formation of white clover, ryegrass and cocksfoot in New Zealand. N.Z. Jl. agric. Res. 20, 337-342.

CARLING, D.E., BROWN, M.F. and BROWN, R.A. (1979). Colonization rates and growth responses of soybean plants infected by vesicular-arbuscular mycorrhizal fungi. Can. J. Bot. 57, 1769-1772.

CHAPMAN, H.D. (1966). Diagnostic criteria for plants and soils. University of California, Div. of Agric. Sciences.

CHARLES, A.H. (1961). Differential survival of cultivars of Lolium, Dactylis and Phleum. J. Br. Grassld. Soc. 16, 69-75.

CHESTNUTT, D.M.B. and LOWE, J. (1970). Agronomy of white clover/grass swards. Review. Br. Grassld Soc. Occ. Symp. 6, 191-213.

CHRISTIE, P., NEWMAN, E.I and CAMPBELL, R. (1978). The influence of neighbouring grassland plants on each others' endomycorrhizas and root-surface micro-organisms. Soil Biol. Biochem. 10, 521-527.

CLAPHAM, A.R., TUTIN, T.G. and WARBURG, E.D. (1962). Flora of the British Isles. 2nd Edition. Cambridge University Press.

COIC, Y. and LESAINT, C. (1971). The equilibrium between potassium and other cations in the organs of higher plants. In: Potassium in biochemistry and physiology. Coll. Int. Potash Inst., 93-103.

COOKE, G.W. (1965). The responses of crops to phosphate fertilizers in relation to soluble phosphorus in soils. MAFF Tech. Bull. 13, 64-74. London, HMSO.

COOPER, K.M. (1973). Mycorrhizal associations of New Zealand ferns. PhD Thesis, University of Otago.

COOPER, K.M. (1975). Growth responses to the formation of endotrophic mycorrhizas in Solanum, Leptospermum and New Zealand ferns. In: Sanders, F.E., Mosse, B. and Tinker, P.B. (eds). Endomycorrhizas. London : Academic Press, 391-407.

COOPER, K.M. and TINKER, P.B. (1978). Translocation and transfer of nutrients in vesicular-arbuscular mycorrhizas. II. Uptake and translocation of phosphorus, zinc and sulphur. New Phytol 81, 43-52.

COPELAND, R. and PATE, J.S. (1969). Nitrogen metabolism of nodulated white clover in the presence and absence of nitrate nitrogen. Br. Grassld. Soc. Occ. Symp. 6, 71-77.

COWIE, G.A. (1951). Potash, its production and place in crop nutrition. London : Edward Arnold & Co.

COX, G., SANDERS, F.E., TINKER, P.B. and WILD, J.A. (1975). Ultrastructural evidence relating to host-endophyte transfer in a vesicular-arbuscular mycorrhiza. In: Sanders, F.E., Mosse, B. and Tinker, P.B. (eds). Endomycorrhizas. London : Academic Press, 297-312.

COX, G. and TINKER, P.B. (1976). Translocation and transfer of nutrients in vesicular-arbuscular mycorrhizas. I. The arbuscule and phosphorus transfer : a qualitative ultrastructural study. New Phytol. 77, 371-378.

CRESS, W.A., THRONEBERRY, G.O and LINDSEY, D.L. (1979). Kinetics of phosphorus absorption by mycorrhizal and non-mycorrhizal tomato roots. Pl. Physiol. 64, 484-487.

CRUSH, J.R. (1973). Significance of endomycorrhizas in tussock grassland in Otago, New Zealand. N.Z. Jl. Bot. 11, 645-660.

CRUSH, J.R. (1974). Plant growth responses to vesicular-arbuscular mycorrhiza. VII. Growth and nodulation of some herbage legumes. New Phytol. 73, 743-749.

CRUSH, J.R. (1975). Occurrence of endomycorrhizas in soils of the Mackenzie Basin, Canterbury, New Zealand. N.Z. Jl. agric. Res. 18, 361-364.

CRUSH, J.R. (1976). Endomycorrhizas and legume growth in some soils of the Mackenzie Basin, Canterbury, New Zealand. N.Z. Jl. agric. Res. 19, 473-476.

CRUSH, J.R. (1978). Changes in effectiveness of soil endomycorrhizal fungal populations during pasture development. N.Z. Jl agric. Res. 21, 683-685.

CULLEN, N.A., McNAUGHT, K.J. and MOUNTIER, N.S. (1965). The establishment of pasture on yellow-brown loams near Te Anau. IV. The effect of lime and phosphate on establishment of clover and grass oversown on uncultivated ground. N.Z. Jl agric. Res. 9, 375-387.

DAFT, M.J., CHILVERS, M.T. and NICOLSON, T.H. (1980). Mycorrhizas of the Liliiflorae. I.Morphogenesis of Endymion nonscriptus (L) Garke and mycorrhizas in nature. New Phytol. 85, 1-10.

DAFT, M.J. and NICOLSON, T.H. (1966). Effect of Endogone mycorrhiza on plant growth. New Phytol. 65, 343-350.

DAFT, M.J. and NICOLSON, T.H. (1969a). Effect of Endogone mycorrhiza on plant growth. II. Influence of soluble phosphate on endophyte and host in maize. New Phytol. 68, 945-952.

DAFT, M.J. and NICOLSON, T.H. (1969b). Effect of Endogone mycorrhiza on plant growth. III. Influence of inoculum concentration on growth and infection in tomato. New Phytol. 68, 953-961.

DAFT, M.J. and NICOLSON, T.H. (1972). Effect of Endogone mycorrhiza on plant growth. IV. Quantitative relationships between the growth of the host and the development of the endophyte in tomato and maize. New Phytol. 71, 287-295.

DAFT, M.J. and Okusanya, B.O. (1973). Effect of Endogone mycorrhiza on plant growth. V. Influence of infection on the multiplication of viruses in tomato, petunia and strawberry. New Phytol. 72, 975-983.

DAFT, M.J. and El GIAHMI, A.A. (1974). Effect of Endogone mycorrhiza on plant growth. VII. Influence of infection on the growth and nodulation in French bean (Phaseolus vulgaris). New Phytol. 73, 1139-1147.

DAFT, M.J. and El GIAHMI, A.A. (1975). Effects of Glomus infection on three legumes. In: Sanders, F.E., Mosse, B. and Tinker, P.B. (eds). Endomycorrhizas. London : Academic Press, 581-592.

DAFT, M.J. and El GIAHMI, A.A. (1978). Effect of arbuscular mycorrhiza on plant growth. VIII. Effects of defoliation and light on selected hosts. New Phytol. 80, 365-372.

DAFT, M.J. and HACSKAYLO, E. (1977). Growth of endomycorrhizal and non-mycorrhizal red maple seedlings in sand and anthracite spoil. Forest Sci. 23, 207-216.

DART, P.J. (1976). Infection and development of leguminous nodules. In: Hardy, R.W.F. and Silver, W.S. (eds). Treatise on dinitrogen fixation. Section III. John Wiley & Sons, 367-472.

DART, P.J., DAY, J.M. and HARRIS, D. (1972). Assay of nitrogenase activity by acetylene reduction. FAO/Int. Atomic Energy Agency Tech. Booklet on Grain Legume Production, 1972.

DART, P.J. and PATE, J.S. (1959). Nodulation studies in legumes. III. The effects of delaying inoculation on the seedling symbiosis of barrel medic, Medicago tribuloides Desr. Aust. J. biol. Sci. 12, 427-444.

DAINTY, J. (1962). Ion transport and electrical potentials in plant cells. Ann. Rev. Pl. Physiol. 13, 379-402.

DAVIES, G.E., NEWBOULD, P. and BAILLIE, G.J. (1979). The effect of controlling bracken (Pteridium aquilinum (L) Kuhn) on pasture production. Grass Forage Sci. 34, 163-171.

DAY, J.M. (1972). Studies on the role of light in legume symbiosis. PhD Thesis, University of London.

DEAL, D.R., BOOTHROYD, C.W. and MAI, W.F. (1972). Replanting of vineyards and its relationship to vesicular-arbuscular mycorrhiza. Phytopathology 62, 172-175.

DEHNE, H.W. and SCHONBECK, F. (1979). Untersuchungen zum Einfluss der endetrophen Mycorrhiza auf Pflanzenkrankheiten. I. Ausbreitung von Fusarium oxysporum f.sp. lycopersici in Tomaten. Phytopath. Z. 95, 105-110.

DEKOCK, P.C. and KIRKBY, E.A. (1969). Uptake by plants of various forms of nitrogen and effects on plant composition. In: Nitrogen and soil organic matter. MAFF Tech. Bull 15, 7-14.

DOWDING, E.S. (1959). Ecology of Endogone. Trans. Br. mycol. Soc. 42, 449-457.

DREW, M.C. (1975). Comparison of the effects of a located supply of phosphate, nitrate, ammonium and potassium on the growth of the seminal root system and the shoot of barley. New Phytol. 75, 479-490.

DUNLOP, J. and BOWLING, D.J.F. (1978a). Uptake of phosphate by white clover. I. Evidence for an electrogenic phosphate pump. J. exp. Bot. 29, 1139-1146.

DUNLOP, J. and BOWLING, D.J.F. (1978b). Uptake of phosphate by white clover. II. The effect of pH on the electrogenic phosphate pump. J. exp. Bot. 29, 1147-1153.

DUNLOP, J., GLASS, A.D.M. and TOMKINS, B.D. (1979). The regulation of K^+ uptake by ryegrass and white clover in roots in relation to their competion for potassium. New Phytol. 83, 365-370.

EADIE, J., ARMSTRONG, R.H., MAXWELL, T.J. and CURRIE, D.C. (1976). Responses in output achieved in improved systems of sheep production. I. In the eastern Cheviots. II. On blanket peat in the west of Scotland. In: Hill land : Proc. Int. Symp., Morgantown, W.Va., USA, 752-756.

El GIAHMI, A.A., NICOLSON, T.H. and DAFT, M.J. (1976). Endomycorrhizal fungi from Libyan soils. Trans. Br. mycol. Soc. 67, 164-169.

ESCA (1963). Grassland management for beef production in the East of Scotland. Tech. Bull. 48, ESCA, 1-7.

ESCA (1977). Fertilizer recommendations. Bull 18.

EVANS, C.C. (1970). X-ray fluorescence analysis for light elements in plant and faecal materials. Analyst 95, 919-929.

EVANS, P.S. (1977). Comparative root morphology of some pasture grasses and clovers. N.Z. Jl agric. Res. 20, 331-335.

FEIGENBAUM, S. and MENGEL, K. (1979). The effect of reduced light intensity and sub-optimal potassium supply on N_2 fixation and N turnover in Rhizobium infected lucerne. Physiologia Pl. 45, 245-249.

FLOATE, M.J.S. (1967). Hill soils. HFRO 4th Rept, 1964-67, 17-21.

FLOATE, M.J.S. (1970). Plant nutrient cycling in hill land. HFRO 5th Rept. 1967-1970, 15-34.

FLOATE, M.J.S. (1977). British hill soil problems. Soil Sci. 123, 325-331.

FLOATE, M.J.S. and PIMPLASKAR, M.S. (1976). Some anomalous results for 'available-P' in hill soils, and experimental attempts twoards their resolution. J. Sci. Fd. Agric. 27, 591.

FLOATE, M.J.S., RANGELEY, A. and BOLTON, G.R. (1980). An investigation of problems of improved pasture production on deep peat, with special reference to potassium responses and interactions with lime and phosphorus. Submitted to Grass Forage Sci.

FORESTRY COMMISSION (1964). Mycorrhizal associations and Calluna heathland afforestation. Forestry Commission Bull. 36.

FOX, J.A. and SPASOFF, L. (1972). Interaction of Heterodera solanacearum and Endogone gigantea on tobacco. J. Nematol. 4, 224-225.

FRED, E.B., BALDWIN, I.L. and McCOY, E. (1972). Root nodule bacteria and leguminous plants. Madison, University of Wisconsin Press, 61-66.

FURLAN, V. and FORTIN, J.A. (1973). Formation of endomycorrhizae by Endogone calospora on Allium cepa under three temperature regimes. Naturaliste Can. 100, 467-477.

FURLAN, V. and FORTIN, J.A. (1977). Effects of light intensity on the formation of vesicular-arbuscular endomycorrhizas on Allium cepa by Gigaspora calospora. New Phytol. 79, 335-340.

GATES, C.T. (1974). Nodule and plant development in Stylosanthes humilis H.B.K. : Symbiotic responses to phosphorus and sulphur. Aust. J. Bot. 22, 45-55.

GERDEMANN, J.W. (1964). The effect of mycorrhiza on the growth of maize. Mycologia 56, 342-349.

GERDEMANN, J.W. (1968). Vesicular-arbuscular mycorrhiza and plant growth. A Rev. Phytopath. 6, 397-418.

GERDEMANN, J.W. (1975). Taxonomy of the Endogonaceae. In: Sanders, F.E., Mosse, B. and Tinker, P.B. (eds). Endomycorrhizas. London : Academic Press, 35-51.

GERDEMANN, J.W. and NICOLSON, T.H. (1962). Endogone spores in cultivated soil. Nature, Lond. 195, 308-309.

GERDEMANN, J.W. and NICOLSON, T.H. (1963). Spores of mycorrhizal Endogone species extracted from soil by wet sieving and decanting. Trans. Br. mycol. Soc. 46, 235-244.

GERDEMANN, J.W. and TRAPPE, J.M. (1974). The Endogonaceae of the Pacific North West. Mycologia Memoir, No. 5

GIBSON, A.H. (1959). A study on the physiology and cytology of nodule formation on members of the family Leguminosae. PhD Thesis. University of London, 100-107.

GIBSON, A.H. (1976). Recovery and compensation by nodulated legumes to environmental stress. In: Nutman, P.S. (ed) Symbiotic nitrogen fixation in plants. IBP 7, 385-403.

GIBSON, A.H. and NUTMAN, P.S. (1960). Studies on the physiology of nodule formation. A reappraisal of the effect of preplanting. Ann. Bot. N.S. 24, 420-433.

GIBSON, A.H. and PAGAN, J.D. (1977). Nitrate effects on the nodulation of legumes inoculated with nitrate-reductase-deficient mutants of Rhizobium. Planta 134, 17-22.

GIBSON, D.I., HAYES, P and LAIDLAW, A.S. (1975). The influence of phosphate and lime on the growth and N fixation of Lotus uliginosus and Trifolium repens under greenhouse conditions. J. Br. Grassld. Soc. 30, 295-301.

GILMORE, A.C. (1968). Phycomycetous mycorrhizal organisms collected by open-pot culture methods. Hilgardia 39, 87-105.

GILMORE, A.E. (1971). The influence of endotrophic mycorrhizae on the growth of peach seedlings. J. Am. Soc. hort. Sci. 96, 35-38.

GRANT, S.A. (1971). The measurement of primary production and utilization on heather moors. J. Br. Grassld. Soc. 26, 51-58.

GRAW, D. (1979). The influence of soil pH on the efficiency of vesicular-arbuscular mycorrhiza. New Phytol. 82, 687-695.

GRAY, L.E. and GERDEMANN, J.W. (1973). Uptake of sulphur-35 by vesicular-arbuscular mycorrhizae. Pl. Soil 39, 687-689.

GRENNAN, E. and MULQUEEN, J. (1964a). Grass production on blanket peat. 1. Phosphorus requirements. Ir. J. agric. Res. 3, 37-48.

GRENNAN, E. and MULQUEEN, J. (1964b). Grass production on blanket peat. 2. Potassium requirements. Ir. J. agric. Res. 3, 51-61.

GRENNAN, E. and MULQUEEN, J. (1964c). Grass production on blanket peat. 3. Nitrogen requirements. Ir. J. agric. Res. 3, 211-222.

GRENNAN, E.J. and O'TOOLE, M.A. (1966). Pasture establishment and maintenance on blanket-peat soil. Proc. 10th Internat. Grassld. Congr. Finland. 842-846.

HAI, T.V. and LAUDELOUT, H. (1966). Absorption of phosphate by rice roots. Ann. Physiol. vég. 8, 13-24.

HAIDOCK, K.P. and NORRIS, D.O. (1967). Opposed curves for nitrogen percent in dry weight by Rhizobium dependent and nitrate dependent legumes. Aust. J. Sci. 29, 426-427.

HALL, D.A. (1971). The influence of varied calcium nutrition on the growth and ionic composition of plants. PhD Thesis, University of Leeds.

HALL, I.R. (1976). Response of Coprosma robusta to different forms of endomycorrhizal inoculum. Trans. Br. mycol. Soc. 67, 409-411.

HALL, I.R. (1977a). Species and mycorrhizal infections of New Zealand Endogonaceae. Trans. Br. mycol. Soc. 68, 341-356.

HALL, I.R. (1977b). Effect of applied nutrients and endomycorrhizas on Metrosideros umbellata and Leptosperum scoparium. N.Z. Jl. Bot. 15, 481-484.

HALL, I.R. (1978a). Effects of endomycorrhizas on the competitive ability of white clover. N.Z. Jl. agric. Res. 21, 509-515.

HALL, I.R. (1978b). Effect of vesibular-arbuscular mycorrhizas on two varieties of maize and one of sweetcorn. N.Z. Jl. agric. Res. 21, 517-519.

HALL, I.R. (1979). Soil pellets to introduce vesicular-arbuscular mycorrhizal fungi into soil. Soil Biol. Biochem. 11, 85-86.

HALL, I.R., SCOTT, R.S. and JOHNSTONE, P.D. (1977). Effect of vesicular-arbuscular mycorrhizas on response of 'Grasslands Huia' and 'Tamar' white clovers to phosphorus. N.Z. Jl. agric. Res. 20, 349-355.

HALLSWORTH, E.G. (1958). Nutritional factors affecting nodulation. In: Hallsworth, E.G. (ed). Nutrition of the Legume. Lond. Butterworths, 183-201.

HARDARSON, G. and JONES, D.GARETH (1979a). The inheritance of preference for strains of Rhizobium trifolii by white clover (Trifolium repens). Ann. appl. Biol. 92, 329-333.

HARDARSON, G. and JONES, D.GARETH (1979b). Effect of temperature on competition amongst strains of Rhizobium trifolii for nodulation of two white clover varieties. Ann. appl. Biol. 92, 229-236.

HARLEY, J.L. (1969). Biology of mycorrhiza. London, Leonard Hill.

HARPER, J.E. and COOPER, R.L. (1971). Nodulation response of soybeans (Glycine max merr.) to application rate and and placement of combined nitrogen. Crop Sci. 11, 438-440.

HATTINGH, M.J. and GERDEMANN, J.W. (1975). Inoculation of Brazilian sour orange seed with an endomycorrhizal fungus. Phytopathology 65, 1013-1016.

HAYMAN, D.S. (1970). Endogone spore numbers in soil and vesicular-arbuscular mycorrhiza in wheat as influenced by season and soil treatment. Trans. Br. mycol. Soc. 54, 53-63.

HAYMAN, D.S. (1974). Plant growth responses to vesicular-arbuscular mycorrhiza. VI. Effect of light and temperature. New Phytol. 73, 71-80.

HAYMAN, D.S. (1975). The occurrence of mycorrhiza in crops as affected by soil fertility. In: Sanders, F.E., Mosse, B. and Tinker, P.B. (eds). Endomycorrhizas. London : Academic Press, 495-509.

HAYMAN, D.S. (1978). Endomycorrhizae. In: Dommergues, Y.R. and Krupa, S.V. (eds). Interactions between non-pathogenic soil microorganisms and plants. Developments in Agricultural and Managed-Forest Ecology 4 : Amsterdam, Elsevier 401-442.

HAYMAN, D.S. and MOSSE, B. (1971). Plant growth responses to vesicular-arbuscular mycorrhiza. I. Growth of Endogone-inoculated plants in phosphate-deficient soils. New Phytol. 70, 19-27.

HAYMAN, D.S. and MOSSE, B. (1972). Plant growth responses to vesicular-arbuscular mycorrhizas. III. Increased uptake of labile P from soil. New Phytol. 71, 41-47.

HAYMAN, D.S. and MOSSE, B. (1979). Improved growth of white clover in hill grasslands by mycorrhizal inoculation. Ann. appl. Biol. 93, 141-148.

HAYSTEAD, A., KING, J. and LAMB, W.I.C. (1979). Photosynthesis respiration and nitrogen fixation in white clover. Grass Forage Sci 34, 125-130.

HAYSTEAD, A. and MARRIOTT, C.A. (1979a). The transfer of legume nitrogen to an associated grass species. Soil Biol. Biochem. 11, 99-104.

HAYSTEAD, A. and MARRIOTT, C.A. (1979b). Effects of different rates and times of application of 'starter' dressings of nitrogen fertiliser to a surface sown perennial ryegrass/ white clover sward on hill peat. Grass Forage Sci. 34, 241-248.

HEPPER, C.M. (1976). A colorimetric method for estimating vesicular-arbuscular mycorrhizal infection in roots. Soil. Biol. Biochem. 9, 15-18.

HEWITT, E.J. and SMITH, T.A. (1975). Plant mineral nutrition. London : English Univ. Press.

HFRO (1979). Science and hill farming. HFRO 1954-1979.

HOLDING, A.J. and KING, J. (1963). The effectiveness of indigenous populations of Rhizobium trifolii in relation to soil factors. Pl. Soil 18, 191-198.

HOLEVAS, C.D. (1966). The effect of vesicular-arbuscular mycorrhiza on the uptake of soil phosphorus by strawberry (Fragaria sp. var. Cambridge Favourite). J Hort. Sci. 41, 57-64.

HIRREL, M.C. and GERDEMANN, J.W. (1979). Enhanced carbon transfer between onions infected with a vesicular-arbuscular mycorrhizal fungus. New Phytol. 83, 731-738.

HUNT, I.V., ALEXANDER, R.H. and RUTHERFORD, A.A. (1964). The effect of various manuring practices on the magnesium status of spring herbage. J. Br. Grassld. Soc. 19, 224-230.

JACKMAN, R.H. and MOUAT, M.C.H. (1972). Competition between grass and clover for phosphate. I. Effect of browntop (Agrostis tenuis Sibth) on white clover (Trifolium repens L.) growth and nitrogen fixation. N.Z. Jl. agric. Res. 15, 653-666.

JACKSON, N.E., FRANKLIN, R.E. and MILLER, R.H. (1972). Effects of vesicular-arbuscular mycorrhizae on growth and phosphorus content of three agronomic crops. Proc. Soil Sci. Soc. Am. 36, 64-67.

JALALI, B.L. and DOMSCH, K.H. (1975). Effect of systemic fungitoxicants on the development of endotrophic mycorrhiza. In: Sanders, F.E., Mosse, B. and Tinker, P.B. (eds) Endomycorrhizas. London : Academic Press, 619-626.

JASPER, D.A., ROBSON, A.D. and ABBOTT, L.K. (1979). Phosphorus and the formation of vesicular-arbuscular mycorrhizas. Soil Biol. Biochem. 11, 501-505.

JOHN, J.A., WOLOCK, F.W. and DAVID, H.A. (1972). Cyclic designs. N.B.S. Applied Mathematics series 62. U.S. Department of Commerce.

JOHNSON, P.N. (1977). Mycorrhizal Endogonaceae in a New Zealand forest. New Phytol. 78, 161-170.

JONES, D.GARETH and HARDARSON, G (1979). Variation within and between white clover varieties in their preference for strains of Rhizobium trifolii. Ann. appl. Biol. 92, 221-228.

JONES, M.B., RUCKMAN, J.E. and LAWLER, P.W. (1972). Critical levels of P in Subclover (Trifolium subterraneum L.). Agron. J. 64, 695-698.

KASPARI, H. (1973). Elektronenmikroskopische Untersuchung zur Feinstrukter der endotrophen Tabakmykorrhiza. Arch. Mikrobiol. 92, 201-207.

KHAN, A.G. (1972). The effect of VA mycorrhizal associations on growth of cereals. I. Effects on maize growth. New Phytol. 71, 613-619.

KHAN, A.G. (1974). The occurrence of mycorrhizas in halophytes, hydrophytes and xerophytes and of Endogone spores in adjacent soils. J. gen. Microbiol. 81, 7-14.

KHAN, A.G. (1975). The effect of VA mycorrhizal associations on growth of cereals. II. Effects on wheat growth. Ann. appl. Biol. 80, 27-36.

KING, J. and NICHOLSON, I.A. (1964). The grasslands of the forest zone. In: The vegetation of Scotland. Burnett, C.H. (ed). Edinburgh : Oliver & Boyd, 168-206.

KIRKBY, E.A. (1969). Ion uptake and ionic balance in plants in relation to the form of nitrogen nutrition. In: Rorison, I.H. (ed). Ecological aspects of the mineral nutrition of plants. Br. Ecol. Soc. Symp. 9, 215-235.

KIRKBY, E.A. (1979). Maximising calcium uptake by plants. Commun. Soil Sci. Pl. Anal. 10, 89-113.

KIRKBY, E.A. and KNIGHT, A.H. (1977). The influence of the level of nitrate nutrition on ion uptake and assimilation, organic acid accumulation and cation-anion balance in whole tomato plants. Pl. Physiol., Lancaster, 60, 349-353.

KLEINSCHMIDT, T.D. and GERDEMANN, J.W. (1972). Stunting of citrus seedlings in fumigated nursery soils related to the absence of endomycorrhiza. Phytopathology 62, 1447-1453.

KRUCKLEMANN, H.W. (1975). Effects of fertilisers, soils, soil tillage and plant species on the frequency of Endogone chlamydospores and mycorrhizal infection in arable soils. In: Sanders, F.E., Mosse, B. and Tinker, P.B. (eds). Endomycorrhizas. London : Academic Press, 511-525.

LAIDLAW, A.S. (1978). Application of nitrogen fertilizer to grass/white clover swards. Agric. N.I. 52, 314-316.

LAMBERT, D.H., BAKER, D.E., and COLE, Jr.H. (1979). The role of mycorrhizae in the interactions of phosphorus with zinc, copper and other elements. Soil Sci. Soc. Am. 43, 976-980.

LANCASTER, L.A. and BALASUBRAMANIAM, R. (1974). An automated procedure for the determination of aluminium in soil and plant digest. J. Sci. Fd. Agric. 25, 381-386.

LANOWSKA, J. (1966). Influence of different sources of nitrogen on the development of mycorrhiza in Pisum sativum. Pam. Pulawski 21, 365-384.

LIE, T.A. (1969). Effect of low pH on different phases of nodule formation in pea plants. Pl. Soil 31, 391-406.

LIEBIG, J. VON (1840). Chemistry and its application to agriculture and physiology. Philadelphia : Peterson.

LOHNIS, M.P. (1960). Effect of magnesium and calcium on the uptake of manganese by various crop plants. Pl. Soil 12, 339-376.

LONERAGAN, J.F. and SNOWBALL, K. (1969). Calcium requirements of plants. Aust. J. agric. Res. 20, 465-478.

LOUGHMAN, R.C. (1969). The uptake of phosphate and its transport within the plant. In: Rorison, J.E. (ed) Ecological aspects of the mineral nutrition of plants. Blackwells, B. ecol. Soc. Symp. 9, 309-322.

LOWTHER, W.L. (1974). Interaction of lime and seed pelleting on the nodulation and growth of white clover. I. Glasshouse trials. N.Z. Jl. agric. Res. 17, 317-323.

LOWTHER, W.L. and LONERAGAN, J.F. (1968). Calcium and nodulation of suberranean clover. Pl. Physiol., Lancaster, 43, 1362-1366.

LUCAS, R.E. and DAVIES, J.F. (1961). Relationships between pH values of organic soils and availabilities of 12 nutrients. Soil Sci. 92, 177-182.

MAAS, C.V. (1969). Calcium uptake by excised maize roots and interactions with alkali cations. Pl. Physiol., Lancaster, 44, 985-989.

MAAS, C.V., MOORE, P.D. and MASON, B.J. (1969). Influence of calcium and magnesium on manganese absorption. Pl. Physiol., Lancaster, 44, 796-800.

McNAUGHT, K.J. (1958). Potassium deficiency in pastures. I. Potassium content of legumes and grasses. N.Z. Jl. agric. Res. 1, 148-181.

McNAUGHT, K.J. (1970). Diagnosis of mineral deficiencies in grass-legume pastures by plant analysis. Norman, M.J.T. (ed). Proc XI Int. Grassld. Cong. Surfers Paradise, Queensland, 334-338.

McNAUGHT, K.J. and DOROFAEFF, F.D. (1960). Magnesium deficiency in white clover. N.Z. Jl. agric. 100, 230.

McNAUGHT, J.J. and DOROFAEFF, F.D. (1965). Magnesium deficiency in pastures. N.Z. Jl. agric. Res. 8, 555-572.

McNAUGHT, K.J. and DURING, C. (1970). Relations between nutrient concentrations in plant tissues and response of white clover to fertilizers on a gley podzol near Westport. N.Z. Jl. agric. Res. 13, 567-590.

McNEUR, A.J. (1953). Effect of soil acidity on growth of pasture plants. I. Pot experiments with grasses and clovers in acidified soils. N.Z. Jl. Sci. Technol. A35, 311-326.

McNEUR, A.J. (1954). Effect of soil acidity on growth of pasture plants. II. A field trial with white clover. N.Z. J. Sci. Technol. A36, 167-176.

MACY, P. (1936). The quantitative mineral nutrient requirements of plants. Pl. Physiol. 11, 749-764.

MAEDA, M. (1954). The meaning of mycorrhiza in regard to systemic botany. Kumamoto J. Sci. B3, 57-84.

MARSCHNER, H. (1974). Calcium nutrition of higher plants. Neth. J. agr. Sci. 22, 275-282.

MARTIN, T.W. (1960). The role of white clover in grassland. Herb. Abs. 30, No. 3, 159-164.

MASTERSON, C.L. and MURPHY, P.M. (1976). Application of the acetylene reduction technique to the study of nitrogen fixation by white clover in the field. In: Nutman, P.S. (ed). Symbiotic nitrogen fixation in plants. IBP 7 Cambridge Univ. Press, 299-316.

MEJSTRIK, V.K. (1965). Study on the development of endotrophic mycorrhizae. In: Plant microbes relationships The Publishing House of Czechoslovak Academy of Science, Prague.

MEJSTRIK, V.K. (1972). Vesicular-arbuscular mycorrhizas of the species of a Molinietum coeruleae L.I. association: the ecolgy. New Phytol. 71, 833-890.

MENGE, J.A., JOHNSON, E.L.V. and MINASSIAN, V. (1979). Effect of heat treatment and three pesticides upon the growth and reproduction of the mycorrhizal fungus Glomus fasciculatus. New Phytol. 82, 473-480.

MENGE, J.A., STEIRLE, D. BAGYARAJ, D.J., JOHNSON, E.L.V. and LEONARD, R.T. (1978a). Phosphorus concentrations in plants responsible for inhibition of mycorrhizal infection. New Phytol. 80, 575-578.

MENGE, J.A., MUNNECKE, D.E., JOHNSON, E.L.V. and CARNES, D.W. (1978b). Dosage response of the vesicular arbuscular mycorrhizal fungi Glomus fasciculatus and G. constrictus to methyl bromide. Phytopath. 68, 1368-1372.

MENGEL, K. (1961). The Donnan distribution of cations in the free space of plant roots and its significance for active cation uptake. Z. Pflanzenernähr. Düng. Bodenk. 95, 240-259.

MENGEL, K., HAGHPARAST, M.R. and KOCH, K. (1974). The effect of potassium on the fixation of molecular nitrogen by root nodules of Vicia faba. Pl. Physiol., Lancaster, 54, 535-538.

MENGEL, K. and KIRKBY, E.A. (1978). Principles of plant nutrition. International Potash Institute, Switzerland.

MOORE, D.R.E. and WAID, J.S. (1971). The influence of washings of living roots on nitrification. Soil Biol. Biochem 3, 69-83.

MOORE, P.D. (1979). Mycorrhizal associations. Nature, Lond. 282, 780.

MOSSE, B. (1957). Growth and chemical composition of mycorrhizal and non-mycorrhizal apples. Nature, Lond. 179, 922-924.

MOSSE, B. (1963). Vesicular-arbuscular mycorrhiza : an extreme form of fungal adaption. In: Nutman, P.S. and Mosse, B. (eds). Symbiotic Associations. Camb. Univ. Press. Symp. Soc. gen. Microbiol. 13, 146-170.

MOSSE, B. (1972). The influence of soil type and Endogone strain on the growth of mycorrhizal plants in phosphate deficient soils. Rev. Ecol. Biol. Sol., T.IX, 529-537.

MOSSE, B. (1973a). Advances in the study of vesicular-arbuscular mycorrhiza. A Rev. Phytopath 11, 171-196.

MOSSE, B. (1973b). Plant growth responses to vesicular-arbuscular mycorrhizas. IV. In soil given additional phosphate. New Phytol. 72, 127-136.

MOSSE, B. (1977a). Plant growth responses to vesicular-arbuscular mycorrhiza. X. Responses of Stylosanthes and maize to inoculation in unsterile soils. New Phytol. 78, 277-288.

MOSSE, B. (1977b). The role of mycorrhiza in legume nutrition on marginal soils. In: Vincent, J.M., Whitney, A.S. and Bose, J. (eds). Exploiting the legume-Rhizobium symbiosis in tropical agriculture. Misc. Publ. Coll. Agric. Univ. Hawaii 145, 275-292.

MOSSE, B. and BOWEN, G.D. (1968). A key to the recognition of some Endogone spore types. Trans. Br. mycol. Soc. 51, 469-483.

MOSSE, B. and HAYMAN, D.S. (1971). Plant growth responses to vesicular-arbuscular mycorrhizas. II. In unsterilised field soils. New Phytol. 70, 29-34.

MOSSE, B., HAYMAN, D.S. and IDE, G.J. (1969). Growth responses of plants in unsterilised soil to inoculation with vesicular-arbuscular mycorrhiza. Nature, Lond. 224, 1031-1032.

MOSSE, B., POWELL, C.Ll. and HAYMAN, D.S. (1976). Plant growth responses to vesicular-arbuscular mycorrhiza. IX. Interactions between VA mycorrhiza, rock phosphate and symbiotic nitrogen fixation. New Phytol. 76, 331-342.

MOUSTAFA, E., BALL, R. and FIELD, T.R.O. (1969). The use of acetylene reduction to study the effect of nitrogen fertilizer and defoliation on nitrogen fixation by field-grown white clover. N.Z. Jl. agric. Res. 12, 691-696.

MUNNS, D.N. (1968). Nodulation of Medicago sativa in solution culture. I. Acid sensitive steps. Pl. Soil 28, 129-146.

MUNNS, D.N. (1970). Nodulation of Medicago sativa in solution culture. V. Calcium and pH requirements during infection. Pl. Soil 32, 90-120.

MUNNS, D.N. (1977a). Mineral nutrition and the legume symbiosis. In: Hardy, R.W.F. and Gibson, A.H. (eds). A treatise on dinitrogen fixation. Section IV. Agronomy and ecology. 353-391.

MUNNS, D.N. (1977b). Soil acidity and related factors. In: Vincent, J.M., Whitney, A.S. and Bose, J. (eds). Exploiting the legume-Rhizobium symbiosis in tropical agriculture. Univ. Hawaii Coll. Trop. Agr. Misc. Publ. 145, 211-236.

MUNNS, D.N. (1978). Soil acidity and nodulation. In: Andrew, C.S. and Kamprath, E.J. (eds). Mineral nutrition of legumes in tropical and subtropical soils. CSIRO, East Melbourne, Vic., Australia 3002, 347-363.

MUNNS, D.N. and FOX, R.L. (1977). Comparative lime requirements of tropical and temperate legumes. Pl. Soil 46, 533-548.

MUNNS, D.N., FOX, R.L. and KOCH, B.L. (1977). Influence of lime on nitrogen fixation by tropical and temperate legumes. Pl. Soil 46, 591-601.

MUNRO, J.M.M. (1969). Objectives of hill land research. J. agric. Soc. Univ. Coll. Wales, Aberyst. 49, 15-19.

MUNRO, J.M.M. (1970). The role of white clover in hill areas. Br. Grassld. Soc. Occ. Symp. No. 6, 259-263.

MUNRO, J.M.M., DAVIES, D.A. and THOMAS, T.A. (1973). Potential pasture production in the uplands of Wales. 3. Soil nutrient resources and limitations. J. Br. Grassld. Soc. 28, 247-255.

MURDOCH, C.L., JACKOBS, J.A. and GERDEMANN, J.W. (1967). Utilisation of phosphorus sources of different availability by mycorrhizal and non-mycorrhizal maize. Pl. Soil 27, 329-334.

MYTTON, L.A. (1975). Plant genotype x Rhizobium strain interactions in white clover. Ann. appl. Biol. 80, 103-107.

NASSERY, H. (1970). Phosphate absorption by plants from habitats of different phosphate status. II. Absorption and incorporation of phosphate by intact plants. New Phytol. 69, 197-203.

NESHEIM, D.N. and LINN, M.B. (1969). Deleterious effects of certain fungitoxicants on the formation of mycorrhiza on corn by Endogone fasciculata and on corn root development. Phytopathol. 59, 297-300.

NEWBOULD, P. (1973). Plants to improve hill pastures. HFRO 6th Rept. 1971-73, 74-85.

NEWBOULD, P. (1974). The improvement of hill pastures for agriculture. A review. Part 1. J. Br. Grassld. Soc. 29, 241-248.

NEWBOULD, P. (1975). Improvement of hill pastures for agriculture. A review. Part 2. J. Br. Grassld Soc. 30, 41-44.

NEWBOULD, P. (1979). The limitations and potential for pasture production in the hills and uplands. BAAS Ann. Meeting.

NEWBOULD, P. and FLOATE, M.J.S. (1979). Problems of hill and upland soils. Welsh Soils Discussion Group (in press).

NEWBOULD, P. and HAYSTEAD, A. (1978). White clover : its role and establishment in hill pastures. HFRO 7th Rept. 1974-77, 49-68.

NEWBOULD, P., HOLDING, A.J., DAVIES, G.J., RANGELEY, A., HAYSTEAD, A., COPEMAN, G., YOUNIE, D., WATERSON, H.A., FRAME, J., HOLMES, J.C., HERRIOTT, J.B.D., DAVIES, M., PARKER, J.W.G., LOWE, J., DAVIES, A. and WILDIG, J. (1980). The effect of Rhizobium inoculation on white clover in improved hill soils in the United Kingdom. In preparation.

NIAB (1978). Recommended varieties of herbage legumes 1978/9. Farmers Leaflet No. 4.

NICHOLSON, T.H. (1967). Vesicular-arbuscular mycorrhiza - a universal plant symbiosis. Sci. Prog., Oxford 55, 561-581.

NSCA (1972). The improvement of hill pastures. College Leaflet No. 9.

NSCA (1978). Fertilizer recommendations. College Bull. No. 15.

NYE, P.H. and TINKER, P.B. (1977). Solute movement in the soil-root systems. Studies in ecology 4. Oxford : Blackwell Scientific Publications.

OGG, W.G. and ROBERTSON, I.M. (1934). The reclamation of moorland. Emp. J. expt. Agric. 2, 163-173.

O'TOOLE, M.A. (1963). Lime requirements for pasture establishment on blanket peat. Int. Peat Congr. Leningrad. Sect. 3, No. 25, 743-753.

O'TOOLE, M.A. (1967). Grassland research on blanket peat. J. Br. Grassld. Soc. 23, 43-50.

O'TOOLE, M.A. (1977). Estimating fertilizer loss on a hill slope. An Foras Taluntais. Res. Rep. 1977. Animal Production, 176-177.

O'TOOLE, M.A. and MASTERSON, C.L. (1968). Interactions of calcium and hydrogen ion concentration on nodulation of white clover in peat. Ir. J. agric. Res. 7, 129-131.

OWUSU-BENNOAH, E. and MOSSE, B. (1979). Plant growth responses to vesicular-arbuscular mycorrhiza. XI. Field inoculation responses in barley, lucerne and onions. New Phytol. 83, 671-679.

OWUSU-BENNOAH, E. and WILD, A. (1979). Autoradiography of the depletion zone of phosphate around onion roots in the presence of vesicular-arbuscular mycorrhiza. New Phytol. 82, 133-140.

PAIRUNAN, A.K., ROBSON, A.D. and ABBOTT, L.K. (1980). The effectiveness of vesicular-arbuscular mycorrhizas in increasing growth and phosphorus uptake of subterranean clover from phosphorus sources of different solubilities. New Phytol. 84, 327-338.

PATE, J.S. and DART, P.J. (1961). Nodulation studies in legumes. IV. The influence of inoculum strain and time of application of ammonium nitrate on symbiotic response. Pl. Soil 15, 329-346.

PEUSS, H. (1958). Untersuchungen zur okologie und bedeutung der Tabakmycorriza. Arch. Mikrobiol. 29, 112-142.

PIMPLASKAR, M., FLOATE, M.J.S. and NEWBOULD, P. (1980). The suitability of different methods for assessing the phosphorus status of hill soils. J. Sci. Fd. Agric. In press.

PORTER, W.M., ABBOTT, L.K. and ROBSON, A.D. (1978). Effect of rate of application of superphosphate on populations of vesicular-arbuscular endophytes. Aust. J. exp. Agric. an. Husb. 18, 573-578.

POWELL, C.Ll. (1975a). Plant growth responses to vesicular-arbuscular mycorrhiza. VIII. Uptake of P by onion and clover infected with different Endogone spore types in ^{32}P labelled soils. New Phytol. 75, 563-566.

POWELL, C.Ll. (1975b). Potassium uptake by endotrophic mycorrhizas. In: Sanders, F.E., Mosse, B. and Tinker, P.B. (eds). Endomycorrhizas. London : Academic Press, 461-468.

POWELL, C.Ll. (1976a). Mycorrhizal fungi stimulate clover growth in New Zealand hill country soils. Nature, Lond. 264, 436-438.

POWELL, C.Ll. (1976b). Development of mycorrhizal infections from Endogone spores and infected root segments. Trans. Br. mycol. Soc. 66, 439-445.

POWELL, C.Ll. (1977a). Mycorrhizas in hill country soils. II. Effect of several mycorrhizal fungi on clover growth in sterilised soils. N.Z. Jl. agric. Res. 20, 59-62.

POWELL, C.Ll. (1977b). Mycorrhizas in hill country soils. III. Effect of inoculation on clover growth in unsterile soils. N.Z. Jl. agric. Res. 20, 343-348.

POWELL, C.Ll. (1979). Inoculation of white clover and ryegrass seed with mycorrhizal fungi. New Phytol. 83, 81-85.

POWELL, C.Ll. and DANIEL, J. (1978). Growth of white clover in undisturbed soils after inoculation with efficient mycorrhizal fungi. N.Z. Jl. agric. Res. 21, 675-681.

POWELL, C.Ll. and SITHAMPARANATHAN, J. (1977). Mycorrhizas in hill country soils. IV. Infection rate in grass and legume species by indigenous mycorrhizal fungi under field conditions. N.Z. Jl. agric. Res. 20, 489-494.

RATNAYAKE, M., LEONARD, R.T. and MENGE, J.A. (1978). Root exudation in relation to supply of phosphorus and its possible relevance to mycorrhizal formation. New Phytol. 81, 543-552.

RAVEN, J.A. and SMITH, F.A. (1976). Nitrogen assimilation and transport in vascular land plants in relation to introcellular pH regulation. New Phytol. 76, 415-431.

RAYNER, M.C. (1927). Mycorrhiza. New Phytol. (Reprint 15), 246.

REDHEAD, J.F. (1975). Endotrophic mycorrhizas in Nigeria: some aspects of the ecology of the endotrophic mycorrhizal association of Khaya grandifoliola C.DC. In: Sanders, F.E., Mosse, B. and Tinker, P.B. (eds). Endomycorrhizas. London : Academic Press, 447-459.

REITH, J.W.S. (1963). The magnesium content of soils and crops. J. Sci. Fd. Agric. 14, 417-426.

REITH, J.W.S. (1973). Soil conditions and nutrient supplies in hill land. Colloquium 3, Potassium Institute Ltd. 5-12.

REITH, J.W.S., INKSON, R.H.E., HOLMES, W., MACLUSKY, D.A., REID, D., HEDDLE, R.G. and COPEMAN, G.J.F. (1964). The effects of fertilizer on herbage production. II. The effect of nitrogen, phosphorus and potassium on botanical composition. J. agric. Sci. Camb. 63, 209-219.

REITH, J.W.S. and ROBERTSON, R.A. (1971). Lime and fertilizer requirements for the establishment and growth of grass on deep peat. J. agric. Sci. Camb. 76, 89-95.

REITH, J.W.S., ROBERTSON, R.A. and INKSON, R.H.E. (1973). The nutrient requirements of herbage on deep acid peat. J. agric. Sci. Camb. 80, 425-434.

RHODES, L.H. and GERDEMANN, J.W. (1975). Phosphate uptake zones of mycorrhizal and non-mycorrhizal onions. New Phytol. 55, 555-561.

RHODES, L.H. and GERDEMANN, J.W. (1978a). Hyphal translocation and uptake of sulphur by vesicular-arbuscular mycorrhizae of onion. Soil Biol. Biochem. 10, 355-360.

RHODES, L.H. and GERDEMANN, J.W. (1978b). Influence of phosphorus nutriton on sulphur uptake by vesicular-arbuscular mycorrhizae of onion. Soil Biol. Biochem. 10, 361-364.

RHODES, L.H. and GERDEMANN, J.W. (1978c). Translocation of calcium and phosphate by external hyphae of vesicular-arbuscular mycorrhizae. Soil Sci. 126, 125-126.

RICE, E.L. (1979). Allelopathy - an update. Bot. Rev. 45, 15-109.

RICHARDSON, H.L. (1938). The nitrogen cycle in grassland soils: with special reference to the Rothamsted Park grass experiment. J. agric. Sci. Camb. 28, 73-121.

RIGAUD, J. (1976). Effet des nitrates sur la fixation d'azote par les nodules de Haricot (Phaseolus vulgaris). Physiol. vég. 14, 297-308.

RILEY, D. and BARBER, S.A. (1971). Effect of ammonium and nitrate fertilization on phosphorus uptake as related to root induced pH changes at the soil/root interface. Proc. Soil Sci. Soc. Am. 35, 301-306.

ROBSON, A.D. and LONERAGAN, J.F. (1978). Responses of pasture plants to soil chemical factors other than nitrogen and phosphorus, with particular emphasis on the legume symbiosis. In: Wilson, J.R. (ed). Plant relations in pastures. Proc. Symp. Brisbane, CSIRO. 1976, 128-142.

ROGERS, J.A. and BRUCE, D. (1977). Allelopathic studies. HFRO. Ann. Rept. 1977.

ROSS, J.P. (1971). Effect of phosphate fertilisation on yield of mycorrhizal and non-mycorrhizal soybeans. Phytopathology 61, 1400-1403.

ROSS, J.P. (1972). Influence of Endogone mycorrhiza on Phytophthora rot in soybean. Phytopathology 62, 896-897.

ROSS, J.P. and GILLIAM, J.W. (1973). Effect of Endogone mycorrhiza on phosphorus uptake by soybeans from inorganic phosphates. Proc. Soil Sci. Soc. Am. 37, 237-239.

ROSS, J.P. and HARPER, J.A. (1970). Effect of Endogone mycorrhiza on soybean yields. Phytopathology 60, 1552-1556.

RUSSELL, E.W. (1973). Soil conditions and plant growth. 10th Edition. Longmans.

RUSSELL, R.S. and BARBER, D.A. (1960). The relationship between salt uptake and the absorption of water by intact plants. A Rev. Pl. Physiol. 11, 127-140.

RUSSELL, R.S. and CLARKSON, D.I. (1976). Ion transport through root systems. In: Sutherland, N. (ed). Perspectives in experimental biology. 2. Botany. Oxford and New York : Pergamon Press, 401-411.

SAC (1979). Classification of grass and clover varieties for Scotland 1979-80. Publication No. 48.

SAFIR, G.R., BOYER, J.S. and GERDEMANN, J.W. (1971). Mycorrhizal enhancement of water transport in soybean. Science, NY 172, 581-583.

SAFIR, G.R., BOYER, J.S. and GERDEMANN, J.W. (1972). Nutrient status and mycorrhizal enhancement of water transport in soybean. Pl. Physiol., Lancaster, 49, 700-703.

SAIF, S.R. and IFFAT, N. (1976). Vesicular-arbuscular mycorrhizae in plants and Endogonaceous spores in the soil of northern areas of Pakistan. Pak. J. Bot. 8, 163-179.

SANDERS, F.E. (1975). The effect of foliar-applied phosphate on the mycorrhizal infections of onion roots. In: Sanders, F.E., Mosse, B. and Tinker, P.B. (eds). Endomycorrhizas. London : Academic Press, 262-276.

SANDERS, F.E. and TINKER, P.B. (1971). Mechanism of absorption of phosphate from soil by Endogone mycorrhizas. Nature, Lond. 233, 278-279.

SANDERS, F.E. and TINKER, P.B. (1973). Phosphate flow in mycorrhizal roots. Pestic. Sci. 4, 385-395.

SANDERS, F.E., TINKER, P.B., BLACK, R.L.B., and PALMERLEY, S.M. (1977). The development of endomycorrhizal root systems. I. Spread of infection and growth promoting effects with four species of vesicular-arbuscular endophyte. New Phytol. 78, 257-268.

SCHENCK, N.C., GRAHAM, S.O. and GREEN, N.E. (1975). Temperature and light effect on contamination and spore germination of vesicular-arbuscular mycorrhizal fungi. Mycologia 67, 1189-1192.

SCHENCK, N.C. and HINSON, K. (1973). Response of nodulating and non-nodulating soybeans to a species of Endogone mycorrhiza. Agron. J. 65, 849-950.

SCHENCK, N.C. and KINLOCH, R.A. (1974). Endotrophic vesicular-arbuscular mycorrhizae on soyabean in Florida. Mycologia 63, 672-674.

SCHENCK, N.C. and SCHRODER, V.N. (1974). Temperature response of Endogone mycorrhiza on soybean roots. Mycologia 66, 600-605.

SCHONBECK, F. and SCHINZER, U. (1972). Untersuchengen über den Einfluss der endotrophen Mycorrhiza auf die TMV-Lasionenbildung in Nicotiana tabacum L. var. Xanthi-n.c. Phytopathol Z. 73, 78-80.

SEARS, P.D., GOODALL, V.C., JACKMAN, R.H. and ROBINSON, G.S. (1965). Pasture growth and soil fertility. 8. The influence of grasses, white clover, fertilizers and the return of herbage clippings on pasture production of an impoverished soil. N.Z. Jl. agric. Res. 8, 270-283.

SHEPPARD, L.J. (1980). Studies of aluminium and phosphorus in hill soils. PhD Thesis, Edinburgh University.

SKIPPER, H.D. and SMITH, G.W. (1979). Influence of soil pH on the soybean - endomycorrhiza symbiosis. Pl. Soil, 53, 559-563.

SIMPSON, K. (1965). The significance of effects of soil moisture and temperature on phosphorus uptake. MAFF Tech. Bull. 13, 19-29.

SMALL, J.G.C. and LEONARD, O.A. (1969). Translocation of C^{14}-labelled photosynthate in nodulated legumes as influenced by nitrate nitrogen. Amer. J. Bot. 56, 187-194.

SMITH, P.F. (1962). Mineral analysis of plant tissues. Ann. Rev. Pl. Physiol. 13, 81-108.

SMITH, S.E. (1974). Mycorrhizal fungi. C.R.C. Critical Reviews in Microbiology 3, 275-313.

SMITH, S.E. and BOWEN, G.D. (1979). Soil temperature, mycorrhizal infection and nodulation of Medicago truncatula and Trifolium subterranean. Soil Biol. Biochem. 11, 469-473.

SMITH, S.E. and DAFT, M.J. (1977). Interactions between growth, phosphate content and nitrogen fixation in mycorrhizal and non-mycorrhizal Medicago sativa. Aust. J. Pl. Physiol. 4, 403-413.

SMITH, S.E., NICHOLAS, D.J.D. and SMITH, F.A. (1979). Effect of early mycorrhizal infection on nodulation and nitrogen fixation in Trifolium subterranean. Aust. J. Pl. Physiol. 6, 305-316.

SMITH, T.F. (1978). Some effects of crop protection chemicals on the distribution and abundance of vesicular-arbuscular endomycorrhizas. J. Aust. Inst. agric. Sci. 44, 82-88.

SNAYDON, R.W. and BRADSHAW, A.D. (1962a). The performance and survival of contrasting natural populations of white clover when planted into an upland Festuca/Agrostis sward. J. Br. Grassld. Soc. 17, 113-118.

SNAYDON, R.W. and BRADSHAW, A.D. (1962b). Differences between natural populations of Trifolium repens in response to mineral nutrients. I. Phosphate. J. exp. Bot. 13, 422-434.

SNEDECOR, G.W. (1965). Statistical methods: applied to experiments in agriculture and biology. 5th Edition. The Iowa State University Press.

SPARLING, G.P. and TINKER, P.B. (1975). Mycorrhizas in Pennine grassland. In: Saunders, F.E., Mosse, B. and Tinker, P.B. (eds). Endomycorrhizas. London : Academic Press. 545-560.

SPARLING, G.P. and TINKER, P.B. (1978a). Mycorrhizal infection in Pennine grassland. I. Levels of infection in the field. J. appl. Ecol. 15, 943-950.

SPARLING, G.P. and TINKER, P.B. (1978b). Mycorrhizal infection in Pennine grassland. II. Effects of mycorrhizal infection on the growth of some upland grasses on γ-irradiated soils. J. appl. Ecol. 15, 951-958.

SPARLING, G.P. and TINKER, P.B. (1978c). Mycorrhizal infection in Pennine grassland. III. Effects of mycorrhizal infection on the growth of white clover. J. appl. Ecol. 15, 959-964.

SPEDDING, C.R.W. and DIEKMAHNS, E.C. (1972). Grasses and legumes in British agriculture. Commonwealth Bureau of Pastures and Field Crops. Bull. 49.

SPRENT, J.I. (1979). The biology of nitrogen-fixing organisms. London : McGraw-Hill.

STAHL, M. (1949). Die Mycorrhiza der Lebermoose mit besonderer berücksichtigung der Thallosen formen. Planta 37, 367-374.

STENUIT, D.F. (1959). Relationship between magnesium content and yield of Belgian soils. Landw. Forsch. 13, 23-29.

STEUCEK, C.G. and KOONTZ, H.V. (1970). Phloem mobility of magnesium. Pl. Physiol., Lancaster, 46, 50-52.

STEWART, W.D.P. (1966). Nitrogen fixation in plants. Univ. Lond. The Athlone Press.

STEWART, A.B. and HOLMES, W.J. (1953). Nitrogenous manuring of grassland. I. Some effects of heavy dressing of nitrogen on the mineral composition. J. Sci. Fd. Agric. 4, 401-408.

STROBEL, G.A. and Mathre, D.E. (1970). Outlines of plant pathology. Van Nostrand Reinhold Co.

SUTCLIFFE, J. (1977). Plants and temperature. Studies in Biology No. 86. Edward Arnold.

SUTTON, J.C. (1973). Development of vesicular-arbuscular mycorrhizae in crop plants. Can. J. Bot. 51, 2487-2493.

SWIFT, G. (1972). A review of factors affecting the mineral content of herbage in relation to animal requirements with particular reference to the north of Scotland. NSCA, Grassland Husbandry Division. Tech. Rept. 1.

TANNER, J.W. and ANDERSON, I.C. (1963). An external effect of inorganic nitrogen in root nodulation. Nature, Lond. 198, 303-304.

THOMPSON, D.J. (1979). Effect of the proportion of legumes in swards on animal output. Br. Grassld. Soc. Occ. Symp. 10, 101-110.

TODD, J.R. (1961). Magnesium in forage plants. I. Magnesium contents of different species and strains as affected by season and soil treatment. J. agric. Sci. Camb. 56, 411-415.

TINKER, P.B. (1975a). Effects of vesicular-arbuscular mycorrhizas on higher plants. In: Symbiosis S.E.B. Symp. 29, 325-349.

TINKER, P.B. (1975b). The soil chemistry of phosphorus and mycorrhizal effects on plant growth. In: Sanders, F.E., Mosse, B. and Tinker, P.B. (eds). Endomycorrhizas. London : Academic Press, 353-371.

TINKER, P.B. (1978). Effects of vesicular-arbuscular mycorrhizas on plant nutrition and plant growth. Physiol. vég. 16, 743-751.

ULRICH, A. (1945). Critical phosphorus and potassium levels in Ladino clover. Proc. Soil Sci. Soc. Amer. 10, 150-161.

ULRICH, A. (1952). Physiological basis for assessing the nutritional requirements of plants. A Rev. Pl. Physiol. 3, 207-228.

VAN DEN HENDE, A., COTTENIE, A. and DE JONGHE, P. (1952). The value of some methods for chemical soil analysis applied to different Belgian soil types. Trans. Meet. Commns. II and IV Int. Soc. Soil Sci. 2, 307-317.

VAN DER KLEY, F.K. (1957). The effect of clovers and herbs on the mineral composition of grass-dominant pasture. Publ. Afd. Grasslandcult. 14, Landbttogesch. Wageningen.

VAN SCHREVEN, D.A. (1958). Some factors affecting the uptake of nitrogen by legumes. In: Hallsworth, E.G. (ed). The nutrition of legumes. London : Butterworths, 137-163.

VERNON, D.M. (1978). The selection of strains of Rhizobium trifolii for inoculation of Trifolium repens for hill pasture. M. Phil. Thesis. University of Edinburgh.

VINCENT, J.M. (1962). Influence of calcium and magnesium on the growth of Rhizobium. J. gen. Microbiol. 28, 653-663.

VINCENT, J.M. (1965). Environmental factors in the fixation of nitrogen by the legume. In: Bartholomew, W.V. and Clark, F.E. (eds). Soil nitrogen. Madison, Am. Soc. Agron. Agronomy 10, 384-435.

VINCENT, J. M. (1970). A manual for the practical study of root nodule bacteria. IBP Handbook No. 15. Oxford : Blackwells Scientific Publications.

VOGEL, A.I. (1961). A text-book of quantitative inorganic analysis including elementary instrumental analysis. 3rd edition. London : Longmans.

WALKER, C. (1979). Hybrid poplar mycorrhizae and endogenacous spores in Iowa. PhD Thesis, Iowa State University.

WALKER, J.C. (1969). Plant pathology. 3rd edition. McGraw Hill.

WALLACE, T. (1961). The diagnosis of mineral deficiencies in plants by visual symptoms. A colour atlas and guide. 3rd edition. London, HMSO.

WHITE, R.E. (1979). Introduction to the principles and practice of soil science. Oxford : Blackwells.

WHITEHEAD, D.C. (1966). Nutrient minerals in grassland herbage. Commonwealth Agricultural Bureaux, Mimeographed Publication No. 1/1966.

WHITELAW, A., ARMSTRONG, R.H., EVANS, C.C. and FAWCETT, A.R. (1979). A study of the effects of copper deficiency in Scottish Blackface lambs on improved hill pasture. Vet. Rec. 104, 455-460.

WILSON, J.K. (1917). Physiological studies of Bacillus radicicola of soybean (Soya max Piper) and factors influencing nodule production. Cornell Agr. Exp. Stn. Bull. 386, 369-413.

WILSON, P.W. (1940). The biochemistry of symbiotic nitrogen fixation. Univ. Wisconsin Press, Madison.

WOLTON, K.M. (1963). Fertilizers and hypomagnesaemia. NAAS Q. Rev. 14, 122-130.

WOODS, F.W. and BROCK, K. (1964). Interspecific transfer of ^{45}Ca and ^{32}P by root systems. Ecology 45, 886-889.

WRIGHT, C.E. (1975). Herbage legumes in Northern Ireland. Ann. Rept. 48, Ag. Inst. Hillsborough, 13-23.

WSAC (1975). Farm fertilizer recommendations. Adv. Publ. 1.

YOUNG, D.J.B. (1958). A study of the influence of nitrogen on the root weight and nodulation of white clover in a mixed sward. J. Br. Grassld. Soc. 13, 106-114.

APPENDICES

APPENDIX 1

Fertilizer applications in the pot experiments in PART I

EXPERIMENT 1 Nitrogen x phosphorus x potassium factorial in two soils

	Application Rate	
	mg/pot[#]	kg/ha
Lime/Calcium		
$CaCO_3$	3000	4323
Ca	1201	1730
Magnesium		
MgO	470	677
Mg	283	408
Nitrogen		
$Ca(NO_3)_2.4H_2O$	0:117:234:469	0:168:337:674
Ca	0: 20: 40: 80	0: 29: 57:114
N	0: 14: 28: 56	0: 20: 40: 80
Phosphorus		
$Ca_3(PO_4)_2$	0:139:278:557	0:200:401:801
Ca	0: 54:108:216	0: 78:155:310
P	0: 28: 56:111	0: 40: 80:160
Potassium		
KCl	0: 53:106:212:424	0: 76:153:305:610
K	0: 28: 56:111:222	0: 40: 80:160:320
Cl	0: 25: 50:100:200	0: 36: 73:145:290

Trace elements see page 45

[#] 10 cm diameter pots

APPENDIX 1 (contd.)

EXPERIMENT 2. The effect of phosphorus and potassium fertilizer on leaf growth

	Application Rate	
	mg/pot[#]	kg/ha
Lime/Calcium		
$CaCO_3$	1200	2715
Ca	480	1087
Magnesium		
$MgSO_4.7H_2O$	45	101
Mg	4	10
S	6	13
Nitrogen	None or 200 ppm N as NH_4NO_3	
Phosphorus		
$Ca_3(PO_4)_2$	0:88:354	0:200:801
Ca	0:34:136	0: 78:310
P	0:18: 71	0: 40:160
Potassium		
KCl	0:34:135	0:76:304
K	0:18: 71	0:40:160
Cl	0:16: 64	0:36:145
Trace Elements	See page 45	

[#] 7.5 cm diameter plant pots

APPENDIX 1 (contd.)

EXPERIMENT 5. The response to magnesium in two soils

	Application Rate	
Lime	mg/pot#	kg/ha
Brown earth		
$CaCO_3$	950	1369
Ca	380	548
Deep peat		
$CaCO_3$	2410	3473
Ca	965	1390
Magnesium		
$MgSO_4.7H_2O$	0: 70:704	0:101:1014
Mg	0: 7: 69	0: 10: 100
S	0: 9: 92	0: 13: 132
Nitrogen	NONE	
Phosphorus		
$Ca_3(PO_4)_2$	521	751
Ca	202	291
P	104	150
Potassium		
K_2SO_4	464	668
K	208	300
S	85	123
Trace elements	see page 45	

10 cm diameter plant pots

APPENDIX 1 (contd.)

EXPERIMENT 6. Lime x magnesium x potassium factorial experiment in peat

	Application Rate	
	mg/pot[#]	kg/ha
Lime		
$CaCO_3$	0:1000:2000:4000	0:1450:2900:5800
Ca	0: 400: 801:1602	0: 580:1160:2320
Magnesium		
$MgSO_4.7H_2O$	0: 250:1000	0: 362:1450
Mg	0: 25: 99	0: 36: 143
S	0: 33: 130	0: 46: 187
Nitrogen	NONE	
Phosphorus		
$Ca_3(PO_4)_2$	500	725
Ca	196	284
P	100	145
Potassium		
KCl	0:100:200:400	0:145:290:580
K	0: 52:105:210	0: 76:152:304
Cl	0: 48: 95:190	0: 70:137:275
Trace elements	see page 45	

[#] 10 cm diameter plant pots

APPENDIX 1 (contd.)

EXPERIMENT 7. The interaction between the form of phosphorus fertilizer and rate of calcium carbonate

	Application Rate mg/pot#	kg/ha
Lime		
$CaCO_3$	0:1000:2000:4000	0:1450:2900:5800
Ca	0: 400: 800:1602	0: 580:1160:2320
Magnesium	NONE	
Nitrogen	NONE	
Phosphorus		
$Ca_3(PO_4)_2$	500:407	725:590
Ca	196: 65	284: 94
P	100:100	145:145
Potassium		
KCl	400	580
K	210	304
Cl	190	275
Trace elements	See page 45	

10 cm diameter plant pots

APPENDIX 2

The yield (g/pot) of White Clover when grown in two soils given four levels of nitrogen, four levels of phosphorus and five levels of potassium fertilizer and harvested twice (Experiment 1)

(a) Sourhope brown earth, dry matter yield of shoots, harvest 1

Nitrogen kg/ha	Potassium kg/ha	Phosphorus kg/ha			
		0	40	80	160
0	0	2.8	2.9	2.7	2.4
	40	2.1	2.9	2.6	3.5
	80	1.7	2.2	2.8	3.7
	160	2.6	2.6	2.7	3.6
	320	2.3	2.0	2.5	2.6
20	0	2.5	2.7	2.4	3.1
	40	1.9	2.2	3.1	3.3
	80	2.3	3.1	3.1	3.5
	160	2.0	3.0	3.4	3.2
	320	1.7	2.7	2.3	3.4
40	0	2.6	1.8	3.4	3.4
	40	2.6	2.3	3.0	3.7
	80	2.1	2.9	3.1	3.0
	160	1.7	2.9	3.1	3.4
	320	1.4	2.3	2.6	3.2
80	0	2.3	2.7	2.4	3.0
	40	2.9	2.3	3.0	3.8
	80	1.6	3.1	3.3	3.3
	160	2.5	2.1	3.3	3.3
	320	1.8	2.5	2.6	2.8

APPENDIX 2 (contd.)

(b) Sourhope brown earth, dry matter yield of shoots, harvest 2

Nitrogen kg/ha	Potassium kg/ha	Phosphorus kg/ha			
		0	40	80	160
0	0	4.1	3.5	3.7	3.4
	40	4.1	4.7	4.5	3.7
	80	4.7	5.1	5.1	5.4
	160	4.8	6.2	6.2	6.3
	320	5.9	4.8	6.5	6.9
20	0	3.7	3.6	3.1	3.2
	40	3.6	4.4	3.9	4.0
	80	5.1	5.8	4.7	4.4
	160	4.3	7.0	6.7	6.0
	320	4.6	6.2	6.7	6.9
40	0	3.6	2.6	3.3	3.0
	40	4.6	4.4	3.9	4.2
	80	5.1	5.3	5.0	5.1
	160	4.3	5.3	6.6	5.6
	320	5.0	6.2	6.7	7.7
80	0	3.9	3.0	3.8	2.8
	40	4.4	4.1	4.8	3.7
	80	3.9	5.4	5.6	4.7
	160	3.9	2.4	6.1	6.9
	320	5.3	7.2	7.1	7.1

APPENDIX 2 (contd.)

(c) Glensaugh dry peat, dry matter yield of shoots, harvest 1

Nitrogen kg/ha	Potassium kg/ha	Phosphorus kg/ha			
		0	40	80	160
0	0	2.4	2.8	3.4	3.5
	40	2.7	6.1	6.6	6.2
	60	2.2	8.8	7.9	9.3
	160	1.5	10.1	12.3	12.5
	320	1.5	9.6	13.4	14.4
20	0	2.8	3.4	3.0	2.9
	40	2.5	5.2	6.5	6.8
	80	0.9	8.0	9.4	8.4
	160	1.4	10.4	12.0	12.3
	320	2.1	10.4	12.3	14.1
40	0	2.6	2.8	2.1	3.1
	40	2.4	6.1	6.3	5.8
	80	0.8	6.7	8.0	8.9
	160	1.8	9.8	12.4	12.8
	320	1.7	9.5	14.0	14.2
80	0	5.3	3.1	4.7	2.9
	40	1.2	5.5	5.7	5.8
	80	1.2	7.2	8.6	8.1
	160	1.8	9.9	11.0	12.5
	320	1.6	10.1	12.3	13.8

APPENDIX 2 (contd.)

(d) Glensaugh dry peat, dry matter yield of shoots, harvest 2

Nitrogen kg/ha	Potassium kg/ha	Phosphorus kg/ha			
		0	40	80	160
0	0	1.7	1.4	1.5	1.0
	40	1.4	3.1	2.7	2.6
	80	1.3	3.5	3.9	3.0
	160	1.3	3.9	3.9	4.0
	320	1.3	3.9	4.1	3.7
20	0	1.2	1.7	1.4	1.3
	40	1.2	3.2	3.0	2.4
	80	1.0	3.3	2.7	2.7
	160	1.2	3.7	3.6	3.4
	320	1.6	3.7	3.8	4.2
40	0	1.3	1.4	1.3	1.4
	40	1.4	3.0	2.8	2.3
	80	1.0	3.3	3.0	2.8
	160	1.3	4.0	3.2	3.7
	320	1.3	3.8	3.9	4.5
80	0	1.4	1.6	1.5	1.3
	40	1.1	2.8	2.4	2.6
	80	1.0	3.6	2.9	2.6
	160	1.2	2.9	3.3	3.3
	320	1.3	3.6	4.9	4.5

APPENDIX 2 (contd.)

(e) Lephinmore deep peat, dry matter yield of shoots, harvest 1

Nitrogen kg/ha	Potassium kg/ha	Phosphorus kg/ha			
		0	40	80	160
0	0	0.3	0.4	0.7	0.7
	40	0.2	2.2	3.0	3.5
	80	0.5	2.3	3.4	4.0
	160	0.1	2.0	3.9	4.2
	320	0.3	1.3	3.6	5.8
20	0	0.2	0.7	0.9	1.1
	40	0.2	1.8	2.7	3.4
	80	0.1	2.0	3.3	4.8
	160	0.3	1.6	3.7	5.0
	320	0.5	1.6	3.2	5.4
40	0	0.2	0.6	1.1	0.8
	40	0.8	2.2	2.7	3.1
	80	0.6	2.0	3.6	4.8
	160	0.3	1.8	3.4	5.2
	320	0.2	1.8	4.0	5.2
80	0	0.1	0.6	0.6	0.5
	40	0.2	2.0	2.8	3.5
	80	0.1	1.9	3.9	4.5
	160	0.2	2.1	3.5	5.4
	320	0.3	1.9	3.4	6.1

APPENDIX 2 (contd.)

(f) Lephinmore deep peat, dry matter yield of shoots, harvest 2

Nitrogen kg/ha	Potassium kg/ha	Phosphorus kg/ha			
		0	40	80	160
0	0	0.6	1.1	0.8	0.9
	40	0.7	1.7	2.5	3.4
	80	0.9	2.1	4.3	5.6
	160	0.9	2.1	4.5	5.1
	320	0.7	2.9	4.2	8.7
20	0	0.5	0.9	0.9	1.0
	40	1.0	1.6	3.2	3.4
	80	0.8	1.9	2.9	4.9
	160	0.7	2.3	3.3	7.5
	320	0.9	2.1	4.2	6.7
40	0	0.7	0.8	1.0	0.8
	40	0.9	1.6	2.8	3.1
	80	0.7	2.0	2.9	5.1
	160	0.9	2.2	3.5	7.3
	320	0.9	2.2	5.3	9.1
80	0	2.2	0.8	1.0	0.7
	40	0.8	1.6	2.7	2.7
	80	0.8	1.8	3.1	5.1
	160	1.1	1.6	3.4	6.4
	320	0.8	2.4	5.4	9.8

APPENDIX 3

The chemical composition of the shoots of White Clover when grown in two soils, given nitrogen, phosphorus and potassium fertilizer treatments and harvested twice (Experiment 1)

(a) Sourhope brown earth, chemical analysis of shoots, % Nitrogen

Harvest	Nitrogen kg/ha	Potassium kg/ha	Phosphorus kg/ha			
			0	40	80	160
1	0	0	4.09	3.56	3.62	4.10
		40	4.05	3.44	3.56	4.11
		80	3.96	3.55	3.46	4.01
		160	3.73	3.47	3.52	4.02
		320	3.87	3.90	3.82	4.18
1	80	0	3.64	3.81	3.96	4.00
		40	3.86	3.66	3.72	3.78
		80	3.58	3.69	3.77	4.08
		160	3.75	3.26	3.86	4.19
		320	3.74	3.81	4.24	4.37
2	0	0	3.24	3.01	2.68	3.55
		40	2.69	2.88	2.96	3.67
		80	2.75	2.81	2.84	3.50
		160	2.64	2.57	2.44	3.20
		320	2.63	3.11	3.05	3.18
2	80	0	3.50	3.55	3.51	3.49
		40	2.78	2.95	2.97	3.58
		80	2.71	2.85	3.27	3.37
		160	2.83	3.26	3.39	3.29
		320	2.70	2.78	2.81	3.17

APPENDIX 3 (contd.)

(b) Sourhope brown earth, chemical analysis of shoots, % Phosphorus

Harvest	Nitrogen kg/ha	Potassium kg/ha	Phosphorus kg/ha			
			0	40	80	160
1	0	0	0.28	0.31	0.31	0.36
		40	0.25	0.26	0.32	0.34
		80	0.29	0.28	0.37	0.32
		160	0.25	0.27	0.28	0.32
		320	0.24	0.27	0.32	0.36
1	80	0	0.30	0.29	0.32	0.36
		40	0.21	0.27	0.33	0.35
		80	0.28	0.28	0.31	0.32
		160	0.23	0.27	0.31	0.35
		320	0.26	0.29	0.28	0.36
2	0	0	0.26	0.24	0.33	0.44
		40	0.19	0.21	0.27	0.38
		80	0.17	0.20	0.22	0.34
		160	0.16	0.17	0.19	0.30
		320	0.13	0.19	0.20	0.26
2	80	0	0.22	0.32	0.32	0.46
		40	0.17	0.21	0.31	0.43
		80	0.17	0.22	0.25	0.35
		160	0.17	0.24	0.22	0.33
		320	0.16	0.15	0.18	0.25

APPENDIX 3 (contd.)

(c) Sourhope brown earth, chemical analysis of shoots, % Potassium

Harvest	Nitrogen kg/ha	Potassium kg/ha	Phosphorus kg/ha			
			0	40	80	160
1	0	0	1.47	1.39	1.31	1.09
		40	2.03	1.74	2.01	1.57
		80	2.95	2.30	2.03	1.91
		160	3.48	3.04	2.88	2.53
		320	3.96	4.04	4.07	4.01
1	80	0	1.61	1.33	1.43	1.07
		40	1.82	1.99	1.90	1.54
		80	2.65	2.16	2.22	1.99
		160	3.10	3.04	2.84	2.96
		320	4.13	3.97	3.83	3.99
2	0	0	0.58	0.51	0.61	0.54
		40	0.73	0.66	0.68	0.65
		80	0.95	0.83	0.73	0.60
		160	1.35	0.98	0.85	0.84
		320	2.03	2.84	1.48	1.67
2	80	0	0.63	0.59	0.52	0.52
		40	0.68	0.79	0.67	0.63
		80	1.04	0.73	0.76	0.66
		160	1.51	0.75	0.89	0.85
		320	2.74	1.61	1.63	1.46

APPENDIX 3 (contd.)

(d) Sourhope brown earth, chemical analysis of shoots, % Calcium

Harvest	Nitrogen kg/ha	Potassium kg/ha	Phosphorus kg/ha			
			0	40	80	160
1	0	0	2.99	2.99	2.67	3.29
		40	2.53	2.91	2.70	2.88
		80	2.42	2.69	2.78	2.73
		160	2.61	2.45	2.81	2.54
		320	2.26	2.32	2.16	2.22
1	80	0	2.75	3.01	2.97	3.49
		40	2.38	2.82	2.73	2.74
		80	2.48	2.72	2.46	2.70
		160	2.50	3.06	2.51	2.25
		320	2.73	2.36	2.22	2.15
2	0	0	3.11	3.14	2.92	3.10
		40	2.70	3.07	2.89	3.09
		80	2.58	3.03	3.02	3.05
		160	2.38	2.63	2.67	2.92
		320	1.99	2.16	2.21	2.40
2	80	0	2.95	3.18	3.15	3.93
		40	2.85	2.88	2.91	2.95
		80	2.41	2.91	3.11	2.94
		160	2.28	2.82	3.08	2.82
		320	1.80	2.10	2.07	2.50

APPENDIX 3 (contd.)

(e) Sourhope brown earth, chemical analysis of shoots, % Magnesium

Harvest	Nitrogen kg/ha	Potassium kg/ha	Phosphorus kg/ha			
			0	40	80	160
1	0	0	0.64	0.67	0.72	0.77
		40	0.61	0.68	0.66	0.64
		80	0.60	0.65	0.68	0.61
		160	0.62	0.61	0.59	0.59
		320	0.55	0.62	0.56	0.57
1	80	0	0.63	0.69	0.65	0.77
		40	0.51	0.66	0.63	0.64
		80	0.57	0.62	0.61	0.62
		160	0.57	0.68	0.60	0.55
		320	0.64	0.54	0.56	0.54
2	0	0	0.68	1.02	0.84	0.70
		40	0.59	0.63	0.64	0.63
		80	0.58	0.61	0.64	0.63
		160	0.50	0.55	0.59	0.60
		320	0.41	0.45	0.47	0.51
2	80	0	0.73	0.71	0.72	0.80
		40	0.59	0.69	0.61	0.63
		80	0.55	0.62	0.63	0.62
		160	0.53	0.72	0.62	0.58
		320	0.42	0.41	0.47	0.51

APPENDIX 3 (contd.)

(f) Lephinmore deep peat, chemical analysis of shoots, % Nitrogen

Harvest	Nitrogen kg/ha	Potassium kg/ha	Phosphorus kg/ha			
			0	40	80	160
1	0	0	2.80	3.57	4.40	4.58
		40	2.76	2.36	2.22	3.49
		80	2.94	2.21	2.26	2.97
		160	2.84	2.25	2.18	2.97
		320	3.16	2.49	2.57	2.92
1	80	0	3.85	4.83	4.71	4.57
		40	3.77	2.18	2.21	2.90
		80	3.85	2.02	1.75	2.96
		160	4.27	2.21	1.91	2.86
		320	3.59	2.42	2.28	2.85
2	0	0	2.09	3.60	4.20	4.37
		40	2.03	2.07	2.69	3.70
		80	2.05	1.99	2.73	3.14
		160	2.11	1.95	2.32	3.21
		320	2.12	1.95	2.27	2.93
2	80	0	3.15	2.32	2.73	2.83
		40	2.76	1.64	2.26	2.50
		80	2.70	1.64	1.86	2.67
		160	2.66	1.79	1.62	2.39
		320	2.35	1.60	1.69	2.24

APPENDIX 3 (contd.)

(g) Lephinmore deep peat, chemical analysis of shoots, % Phosphorus

Harvest	Nitrogen kg/ha	Potassium kg/ha	Phosphorus kg/ha			
			0	40	80	160
1	0	0	0.11	0.18	0.27	0.40
		40	0.09	0.10	0.12	0.25
		80	0.08	0.09	0.11	0.20
		160	0.09	0.09	0.10	0.22
		320	0.10	0.09	0.11	0.16
1	80	0	0.11	0.18	0.28	0.38
		40	0.10	0.08	0.13	0.18
		80	0.12	0.08	0.10	0.20
		160	0.12	0.08	0.09	0.16
		320	0.11	0.09	0.10	0.16
2	0	0	0.09	0.24	0.32	0.40
		40	0.08	0.12	0.17	0.33
		80	0.08	0.10	0.19	0.30
		160	0.09	0.09	0.15	0.32
		320	0.09	0.09	0.16	0.25
2	80	0	0.12	0.19	0.29	0.48
		40	0.09	0.11	0.19	0.22
		80	0.10	0.10	0.15	0.29
		160	0.10	0.09	0.12	0.25
		320	0.10	0.09	0.14	0.23

APPENDIX 3 (contd.)

(h) Lephinmore deep peat, chemical analysis of shoots, % Potassium

Harvest	Nitrogen kg/ha	Potassium kg/ha	Phosphorus kg/ha			
			0	40	80	160
1	0	0	0.58	0.30	0.30	0.31
		40	2.18	0.74	0.53	0.48
		80	2.67	1.32	0.84	0.58
		160	3.08	2.67	1.44	0.63
		320	3.62	3.23	3.04	2.20
1	80	0	0.77	0.35	0.31	0.34
		40	2.37	0.77	0.54	0.41
		80	3.88	1.43	0.80	0.58
		160	3.78	2.71	1.77	1.07
		320	3.94	2.93	2.81	2.05
2	0	0	0.42	0.28	0.28	0.27
		40	1.37	0.49	0.39	0.35
		80	2.33	0.73	0.46	0.39
		160	2.70	1.43	0.69	0.40
		320	3.10	2.37	1.07	0.74
2	80	0	0.50	0.37	0.29	0.31
		40	1.68	0.60	0.42	0.43
		80	2.45	0.95	0.56	0.45
		160	2.85	1.82	0.89	0.56
		320	3.19	2.68	1.37	0.73

APPENDIX 3 (contd.)

(i) Lephinmore deep peat, chemical analysis of shoots, % Calcium

Harvest	Nitrogen kg/ha	Potassium kg/ha	Phosphorus kg/ha			
			0	40	80	160
1	0	0	1.90	2.93	2.67	3.06
		40	1.59	1.84	2.50	3.73
		80	1.43	1.65	1.97	3.89
		160	1.44	1.42	1.66	3.73
		320	1.51	1.44	1.85	2.20
1	80	0	2.11	2.78	3.37	3.31
		40	1.99	1.69	2.73	3.25
		80	1.95	1.54	2.01	3.80
		160	1.96	1.37	1.47	2.70
		320	1.57	1.29	1.51	2.52
2	0	0	2.39	3.00	2.89	2.65
		40	1.86	2.63	3.43	3.23
		80	1.56	2.41	3.55	3.31
		160	1.69	2.07	3.25	3.21
		320	1.54	1.87	3.34	3.18
2	80	0	2.96	3.24	3.60	3.43
		40	2.06	2.56	4.03	3.41
		80	2.16	2.48	3.39	3.83
		160	2.10	2.06	2.55	3.55
		320	1.83	1.83	2.70	3.20

APPENDIX 3 (contd.)

(j) Lephinmore deep peat, chemical analysis of shoots, % Magnesium

Harvest	Nitrogen kg/ha	Potassium kg/ha	Phosphorus kg/ha			
			0	40	80	160
1	0	0	0.42	0.81	0.86	1.00
		40	0.35	0.40	0.56	0.91
		80	0.31	0.36	0.43	0.85
		160	0.31	0.28	0.36	0.79
		320	0.33	0.29	0.39	0.51
1	80	0	0.44	0.65	0.86	0.81
		40	0.38	0.32	0.58	0.69
		80	0.39	0.31	0.39	0.77
		160	0.39	0.27	0.30	0.54
		320	0.40	0.27	0.30	0.49
2	0	0	0.68	1.16	1.25	1.31
		40	0.45	0.64	0.99	1.18
		80	0.37	0.57	0.81	0.99
		160	0.43	0.46	0.73	1.02
		320	0.40	0.43	0.71	0.72
2	80	0	0.71	1.13	1.32	1.27
		40	0.46	0.59	0.92	1.04
		80	0.47	0.53	0.80	0.91
		160	0.46	0.44	0.53	0.78
		320	0.45	0.40	0.53	0.61

APPENDIX 4

The values for leaf area calculated from the length of the central leaflet
(Experiment 2)

Length of central leaflet (mm)	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17
Leaf area (cm^2)	0.2	0.4	0.8	1.0	1.2	1.4	1.7	1.9	2.3	2.8	3.2	3.5	3.9	4.4	5.2

APPENDIX 5

The total number of leaves, the number, weight and length of green leaves of white clover given phosphorus and potassium when treated with mycorrhizal fungi and mineral nitrogen (Experiment 2)

Fertilizer Treatment	Non-mycorrhizal Fixed Nitrogen	Non-mycorrhizal Mineral Nitrogen	Mycorrhizal Fixed Nitrogen	Mycorrhizal Mineral Nitrogen
(a) Total number of leaves (No./10 pl)				
P0 K0	3	4	13	18
P40 K40	22	28	35	56
P40 K160	16	22	28	54
P160K40	59	95	75	103
P160K160	87	133	95	136
(b) Weight of green leaves (mg/10 pl)				
P0 K0	3	3	121	120
P40 K40	239	267	463	618
P40 K160	125	164	445	490
P160 K40	463	612	582	704
P160 K160	845	1176	897	1836
(c) Number of green leaves (No./10 pl)				
P0 K0	2	3	10	14
P40 K40	19	24	34	52
P40 K160	13	17	27	50
P160 K40	41	58	52	69
P160 K160	79	108	89	130
(d) Length of the central leaflet (mm)				
P0 K0	5.0	4.4	9.4	9.6
P40 K40	10.8	10.4	12.0	11.6
P40 K160	10.4	10.0	12.0	11.2
P160 K40	12.0	12.0	12.8	11.6
P160 K160	13.4	13.6	14.0	13.4

APPENDIX 6

The dry matter yield (kg/ha) of herbage sampled on two occasions, given different levels of phosphorus and potassium fertiliser and grown on deep peat at the Lephinmore Research Station (Experiment 4)

	HARVEST DATE	TREATMENT							
		P0K0	P0K50	P30K0	P30K50	P30K100	P40K50	P40K100	P50K150
T. repens	14/6/78	106	69	224	265	266	195	303	217
	22/8/78	385	445	298	1340	2024	1287	1961	2475
L. perenne	14/6/78	77	75	128	110	117	103	114	148
	22/8/78	169	209	396	448	335	399	326	430
Indigenous species	14/6/78	134	115	87	120	115	73	121	106
	22/8/78	404	386	332	281	215	288	270	188
TOTAL	14/6/78	317	259	439	495	498	371	538	471
	22/8/78	958	1040	1026	2069	2574	1974	2557	3093

APPENDIX 7

The number of seedlings established when white clover was grown in peat and given four levels of lime, three levels of magnesium and four levels of potassium fertilizer (Experiment 6)

Harvest	Calcium carbonate kg/ha	Magnesium kg/ha	Potassium kg/ha			
			0	76	152	304
1	0	0	25	33	34	31
		36	35	34	36	34
		143	33	35	35	33
1	1450	0	32	38	35	34
		36	35	34	37	36
		143	34	36	35	33
1	2900	0	36	33	37	34
		36	37	36	35	35
		143	35	35	35	35
1	5800	0	29	35	36	34
		36	35	36	35	36
		143	34	35	37	34

APPENDIX 8

The yield (g/pot) when white clover was grown in peat and given four levels of lime, three levels of magnesium and four levels of potassium fertilizer, and harvested three times (Experiment 5)

Harvest	Calcium Carbonate kg/ha	Magnesium kg/ha	Potassium kg/ha			
			0	76	152	304
1	0	0	0.6	1.6	2.0	2.0
		36	0.6	1.4	0.7	2.4
		143	0.7	1.9	2.2	2.6
1	1450	0	1.1	2.6	2.3	2.4
		36	1.3	2.5	3.2	2.8
		143	1.3	2.3	2.5	2.8
1	2900	0	1.1	1.7	2.0	2.0
		36	1.1	2.0	2.2	1.9
		143	1.0	1.8	1.8	1.7
1	5800	0	0.5	0.6	1.0	0.9
		36	0.8	0.8	0.9	0.8
		143	0.7	0.9	0.7	0.4
2	0	0	0.2	0.7	0.9	1.0
		36	0.3	0.9	1.1	0.9
		143	0.3	0.7	1.0	0.6
2	1450	0	0.6	2.1	3.3	5.7
		36	0.6	2.5	3.7	6.2
		143	0.7	2.4	4.0	6.1
2	2900	0	0.7	2.6	3.7	5.6
		36	0.9	3.0	4.5	6.3
		143	0.7	3.0	4.8	5.7
2	5800	0	0.8	2.6	3.4	4.3
		36	0.9	2.7	3.6	3.7
		143	0.8	3.1	3.4	3.1
3	0	0	0.1	0.2	0.3	0.3
		36	0.1	0.2	0.5	0.3
		143	0.2	0.2	0.3	0.2
3	1450	0	0.3	1.1	2.1	3.4
		36	0.4	1.7	1.9	3.7
		143	0.4	1.4	2.1	3.4
3	2900	0	0.5	1.8	2.7	5.3
		36	0.6	2.0	2.9	6.3
		143	0.4	1.9	3.5	6.7
3	5800	0	0.7	2.4	3.1	3.0
		36	0.6	2.6	3.6	5.2
		143	0.6	3.0	4.6	6.7

APPENDIX 9

The chemical analysis of shoots when white clover was grown in peat and given four levels of lime, three levels of magnesium and four levels of potassium fertilizer, and harvested three times. (Experiment 5)

(a) % Nitrogen in the shoots

Harvest	Calcium carbonate kg/ha	Magnesium kg/ha	Potassium kg/ha			
			0	76	152	304
1	0	0	5.18	4.00	3.10	2.86
		36	4.74	3.71	3.43	2.45
		143	4.49	2.85	2.11	3.00
1	1450	0	4.57	3.94	3.63	3.60
		36	4.34	3.92	3.99	3.41
		143	4.01	3.78	3.92	3.56
1	2900	0	4.12	3.82	3.87	3.79
		36	3.81	3.65	3.74	3.65
		143	3.81	3.82	3.69	3.73
1	5800	0	4.37	4.82	4.44	4.40
		36	4.31	4.54	4.84	4.78
		143	4.45	4.74	4.74	4.74
2	0	0	4.64	2.60	2.31	1.41
		36	3.77	1.88	1.63	1.19
		143	3.69	1.81	1.53	1.61
2	1450	0	4.54	4.52	3.77	3.65
		36	4.98	4.35	4.40	3.35
		143	4.80	4.37	4.09	3.76
2	2900	0	4.23	3.81	3.23	2.75
		36	3.86	3.86	3.25	3.06
		143	4.64	3.92	3.51	3.41
2	5800	0	3.89	2.85	2.82	2.71
		36	4.43	3.00	2.89	2.84
		143	4.23	3.10	3.02	2.91
3	0	0	2.63	1.55	1.84	1.68
		36	2.14	1.78	1.11	1.30
		143	2.56	1.57	1.51	1.03
3	1450	0	3.88	4.21	3.25	3.52
		36	3.89	3.93	3.67	3.47
		143	3.82	3.82	3.65	3.61
3	2900	0	4.21	3.71	3.54	3.19
		36	3.93	3.84	3.44	3.33
		143	4.16	3.84	3.64	3.13
3	5800	0	3.73	2.54	2.08	2.00
		36	4.09	2.74	2.28	1.99
		143	4.05	3.13	2.54	2.12

APPENDIX 9 (contd.)

(b) % Phosphorus in the shoots

Harvest	Calcium carbonate kg/ha	Magnesium kg/ha	Potassium kg/ha			
			0	76	152	304
1	0	0	0.78	0.56	0.46	0.39
		36	0.63	0.45	0.43	0.35
		143	0.71	0.56	0.42	0.40
1	1450	0	0.60	0.40	0.38	0.33
		36	0.55	0.37	0.39	0.34
		143	0.57	0.41	0.37	0.36
1	2900	0	0.44	0.32	0.26	0.25
		36	0.42	0.31	0.28	0.26
		143	0.42	0.31	0.28	0.27
1	5800	0	0.31	0.31	0.28	0.25
		36	0.35	0.29	0.28	0.27
		143	0.35	0.27	0.29	0.28
2	0	0	0.87	0.67	0.46	0.37
		36	0.68	0.43	0.34	0.39
		143	0.77	0.50	0.41	0.49
2	1450	0	0.53	0.41	0.36	0.29
		36	0.55	0.42	0.38	0.31
		143	0.56	0.41	0.36	0.31
2	2900	0	0.46	0.28	0.25	0.21
		36	0.46	0.27	0.27	0.24
		143	0.45	0.28	0.28	0.23
2	5800	0	0.32	0.22	0.18	0.17
		36	0.33	0.19	0.20	0.19
		143	0.34	0.22	0.19	0.22
3	0	0	0.92	0.83	0.62	0.64
		36	0.82	0.75	0.65	0.71
		143	1.16	1.01	0.88	0.82
3	1450	0	0.54	0.47	0.45	0.33
		36	0.53	0.51	0.44	0.35
		143	0.62	0.55	0.47	0.37
3	2900	0	0.43	0.31	0.25	0.21
		36	0.41	0.34	0.33	0.23
		143	0.42	0.35	0.36	0.27
3	5800	0	0.28	0.14	0.12	0.11
		36	0.33	0.16	0.14	0.11
		143	0.33	0.18	0.17	0.12

APPENDIX 9 (contd.)

(c) % Potassium in the shoots

Harvest	Calcium carbonate kg/ha	Magnesium kg/ha	Potassium kg/ha			
			0	76	152	304
1	0	0	0.47	2.13	2.97	4.33
		36	0.71	1.66	3.20	3.91
		143	0.59	1.60	2.57	2.88
1	1450	0	0.40	1.35	2.39	3.51
		36	0.39	1.35	1.94	3.21
		143	0.35	1.53	2.19	3.28
1	2900	0	0.44	1.85	2.55	3.30
		36	0.37	1.62	2.44	3.21
		143	0.44	1.73	2.50	3.46
1	5800	0	0.82	3.45	3.53	3.92
		36	0.94	2.93	3.62	2.68
		143	0.91	3.63	3.89	4.31
2	0	0	0.41	1.31	1.74	3.49
		36	0.36	1.29	3.30	2.98
		143	0.39	1.07	1.66	3.01
2	1450	0	0.33	0.80	1.35	2.16
		36	0.36	0.81	1.05	1.90
		143	0.30	0.88	1.24	2.03
2	2900	0	0.34	0.94	1.39	2.37
		36	0.31	0.86	1.35	2.35
		143	0.36	0.96	1.55	2.45
2	5800	0	0.53	1.88	1.98	3.09
		36	0.41	1.30	2.07	3.36
		143	0.54	1.43	2.47	3.87
3	0	0	0.42	1.20	1.74	2.75
		36	0.41	0.99	1.87	3.31
		143	0.38	0.90	1.28	3.09
3	1450	0	0.22	0.28	0.33	0.49
		36	0.21	0.27	0.29	0.44
		143	0.22	0.24	0.26	0.33
3	2900	0	0.21	0.25	0.30	0.48
		36	0.20	0.23	0.27	0.37
		143	0.19	0.22	0.25	0.36
3	5800	0	0.18	0.31	0.47	0.57
		36	0.19	0.27	0.39	0.51
		143	0.19	0.24	0.27	0.72

APPENDIX 9 (contd.)

(d) % Calcium in the shoots

Harvest	Calcium carbonate kg/ha	Magnesium kg/ha	Potassium kg/ha			
			0	76	152	304
1	0	0	1.16	1.21	1.02	0.84
		36	1.32	1.04	1.01	0.75
		143	1.31	1.18	0.86	1.43
1	1450	0	3.42	2.53	2.57	2.24
		36	3.29	2.59	2.19	2.04
		143	2.88	2.19	1.99	1.75
1	2900	0	4.32	3.03	2.97	2.71
		36	4.17	3.07	2.91	2.80
		143	4.15	2.88	2.93	2.53
1	5800	0	3.48	3.22	2.77	2.49
		36	5.04	3.27	2.46	2.10
		143	3.67	2.45	2.81	2.59
2	0	0	1.26	1.51	1.34	1.05
		36	1.14	1.18	0.77	0.99
		143	1.38	1.24	1.04	1.17
2	1450	0	3.11	2.83	2.70	2.44
		36	2.89	2.66	2.53	2.41
		143	2.61	2.40	2.16	2.11
2	2900	0	3.89	3.59	3.46	2.81
		36	3.97	3.56	3.59	3.21
		143	3.51	3.12	3.30	2.90
2	5800	0	3.45	2.82	2.38	2.00
		36	3.45	2.95	2.73	2.17
		143	3.51	2.99	2.08	2.11
3	0	0	1.79	2.58	2.27	2.39
		36	1.91	2.00	1.52	1.86
		143	1.82	1.70	1.54	1.89
3	1450	0	3.44	2.69	2.66	2.10
		36	3.21	2.64	2.55	2.07
		143	3.07	2.59	2.29	1.96
3	2900	0	3.41	3.60	3.51	2.84
		36	3.19	3.46	3.54	2.92
		143	3.02	3.14	2.98	2.92
3	5800	0	3.65	4.09	3.92	3.54
		36	3.17	4.08	3.71	3.74
		143	3.03	3.75	4.24	3.46

APPENDIX 9 (contd.)

(e) % Magnesium in the shoots

Harvest	Calcium carbonate kg/ha	Magnesium kg/ha	Potassium kg/ha			
			0	76	152	304
1	0	0	0.43	0.32	0.28	0.22
		36	0.68	0.42	0.48	0.37
		143	1.12	1.02	0.78	0.79
1	1450	0	0.34	0.22	0.23	0.20
		36	0.57	0.38	0.34	0.34
		143	1.02	0.72	0.67	0.62
1	2900	0	0.38	0.22	0.21	0.20
		36	0.52	0.33	0.32	0.29
		143	0.84	0.63	0.59	0.48
1	5800	0	0.33	0.25	0.22	0.19
		36	0.46	0.32	0.27	0.27
		143	0.65	0.34	0.43	0.44
2	0	0	0.46	0.32	0.25	0.22
		36	0.51	0.44	0.34	0.40
		143	1.08	1.14	0.93	1.11
2	1450	0	0.30	0.21	0.18	0.19
		36	0.55	0.36	0.32	0.27
		143	0.93	0.81	0.73	0.63
2	2900	0	0.32	0.24	0.17	0.16
		36	0.53	0.37	0.31	0.26
		143	0.74	0.63	0.60	0.45
2	5800	0	0.34	0.20	0.16	0.14
		36	0.46	0.28	0.26	0.22
		143	0.59	0.44	0.34	0.35
3	0	0	0.52	0.58	0.51	0.52
		36	0.92	1.00	0.65	0.90
		143	2.25	2.33	2.18	2.12
3	1450	0	0.49	0.24	0.17	0.07
		36	0.82	0.50	0.38	0.23
		143	1.44	1.24	0.91	0.58
3	2900	0	0.41	0.18	0.11	0.07
		36	0.63	0.38	0.29	0.15
		143	0.96	0.89	0.64	0.51
3	5800	0	0.41	0.21	0.13	0.12
		36	0.62	0.34	0.29	0.21
		143	0.81	0.69	0.60	0.40

APPENDIX 10

The dry weight of roots, the number of nodules and the pH of the soil at the third harvest when white clover was grown in peat and given four levels of lime, three levels of magnesium and four levels of potassium fertilizer (Experiment 5)

Harvest	Calcium carbonate kg/ha	Magnesium kg/ha	Potassium kg/ha			
			0	76	152	304
(a)	Dry wt.of the roots (g/pot)					
3	0	0	0.19	0.42	0.58	0.67
		36	0.24	0.63	0.34	0.78
		143	0.22	0.53	0.57	0.64
3	1450	0	0.20	0.59	0.99	1.30
		36	0.28	0.75	1.05	1.45
		143	0.31	0.66	1.03	1.55
3	2900	0	0.29	0.81	1.11	2.09
		36	0.35	0.81	1.17	2.42
		143	0.27	0.65	1.08	2.40
3	5800	0	0.35	1.31	2.00	2.35
		36	0.23	1.46	1.86	2.60
		143	0.31	1.33	1.64	2.07
(b)	Number of nodules (No./pot)					
3	0	0	2	0	0	0
		36	0	0	0	0
		143	0	0	0	0
3	1450	0	22	72	127	124
		36	48	92	132	126
		143	56	80	162	156
3	2900	0	42	200	126	216
		36	112	122	226	240
		143	76	186	270	230
3	5800	0	104	196	158	198
		36	94	282	214	272
		143	94	260	278	406
(c)	pH of the soil					
3	0	0	3.66	3.76	3.52	3.32
		36	3.70	3.53	3.43	3.37
		143	3.59	3.61	3.37	3.39
3	1450	0	4.18	4.07	4.02	4.07
		36	4.15	4.01	3.99	4.00
		143	4.00	3.99	3.92	3.81
3	2900	0	4.92	4.75	4.52	4.42
		36	4.77	4.59	4.38	4.20
		143	4.77	4.62	4.29	4.02
3	5800	0	5.94	5.61	5.47	5.37
		36	5.76	5.57	5.43	5.14
		143	5.93	5.42	5.33	5.13

APPENDIX 11

The number of seedlings established, the yield of shoots and roots and the nodules of white clover given four levels of calcium carbonate and two forms of phosphorus (Experiment 8)

Measurement	Calcium carbonate	$Ca(H_2PO_4)_2H_2O$	$Ca_3(PO_4)_2$
Seedlings established	0	8	30
(100 days after	1450	35	37
sowing)	2900	38	39
	5800	37	37
Dry matter yield	0	0.1	2.9
and shoot	1450	3.8	3.9
(g/pot)	2900	3.6	2.3
	5800	1.9	0.6
Dry matter yield of	0	0.5	0
root	1450	0.7	0.7
(g/pot)	2900	0.5	0.7
	5800	0.2	0.4
Number of nodules	0	0	0
(No./pot)	1450	310	400
<1 mm	2900	1481	587
	5800	270	686
>1 mm	0	0	0
	1450	109	128
	2900	101	49
	5800	22	1

APPENDIX 12

Fertilizer applications given in mycorrhizal pot experiments - amounts applied to each pot and the equivalents in kg/ha

EXPERIMENT 8. Inoculation of white clover with indigenous endophytes in non-sterile peat with two levels of added phosphorus

	Application Rate	
	mg/pot #	kg/ha
Lime		
$CaCO_3$	2000	2900
Ca	801	1160
Magnesium	NONE	
Nitrogen	NONE	
Phosphorus		
$Ca_3(PO_4)_2$	139:557	200:801
Ca	54:216	78:310
P	28:111	40:160
Potassium		
KCl	212	305
K	111	160
Cl	100	145
Trace elements	see page 45	

10 cm diameter pots

EXPERIMENT 9. The effect of *G. mosseae* L1, form of nitrogen, phosphorus and potassium fertilizers on the growth of white clover in peat

See Appendix 1, experiment 2

EXPERIMENT 10. Indigenous endophytes in three soils

Plants were watered with quarter strength Dart and Pate solution plus nitrogen but without phosphate (Dart and Pate, 1959). K_2HPO_4 was replaced with KCl to provide the same amount of potassium.

APPENDIX 12 (contd.)

EXPERIMENT 11. The effect of *G. mosseae* L1 and *G. fasciculatus* E3 and Gafsa phosphate on the growth of two cultivars of white clover in peat

EXPERIMENT 12. The effect of *G. mosseae* L1 on the growth of white clover in the brown earth from Sourhope

	Application Rate	
	mg/pot[#]	kg/ha
Lime		
Peat (Expt. 11)		
$CaCO_3$	1200	2715
Ca	480	1087
Brown earth (Expt. 12)		
$CaCO_3$	300	678
Ca	120	272
Magnesium	None	
Nitrogen	None	
Phosphorus		
Gafsa	45	102
P	6	13
Potassium		
KCl	50	113
K	26	59
Cl	24	54
Trace elements	See page 45	

[#] 7.5 cm diameter plant pots

APPENDIX 12 (contd.)

EXPERIMENT 13. The effect of four endophytes and superphosphate on the growth of white clover in the brown earth from Sourhope

	Application Rate	
	mg/pot#	kg/ha
Lime/Calcium		
$CaCO_3$	2760	4000
Ca	1104	1602
Magnesium	None	
Nitrogen	None	
Phosphorus		
Superphosphate	0:35	0:50
Potassium		
KCl	85	190
K	45	100
Cl	40	90
Trace elements	See page 45	

10 cm diameter pots

APPENDIX 13

The dry weight of green leaves (mg/10 pl) taken from white clover, at eight successive harvests, when treated with a mycorrhizal fungus and nitrogen, phosphorus and potassium fertilizers (Experiment 9)

Harvest		1	2	3	4	5	6	7	8
Growth Period (days from sowing)		0-35	35-48	48-62	62-76	76-90	90-123	123-151	151-193
Length of Growth Period (days)		35	13	14	14	14	33	28	42
Treatment		Uninoculated - Fixed Nitrogen							
P0	K0	9	3	4	3	4	2	1	0
P40	K40	123	239	332	312	289	330	258	27
P40	K160	56	125	241	464	673	247	367	41
P160	K40	755	463	318	250	162	132	92	11
P160	K160	912	845	643	495	410	250	138	20
		Uninoculated - Mineral Nitrogen							
P0	K0	8	3	1	1	0	0	0	0
P40	K40	105	267	349	341	191	134	68	3
P40	K160	76	164	273	388	241	73	165	14
P160	K40	1547	612	345	257	127	28	0	0
P160	K160	1551	176	710	387	155	23	2	0
		Inoculated with mycorrhizal fungi - Fixed Nitrogen							
P0	K0	69	121	180	140	177	148	96	11
P40	K40	451	463	367	371	338	294	170	31
P40	K160	217	445	521	620	767	512	531	61
P160	K40	884	582	359	296	283	203	130	15
P160	K160	912	897	943	703	551	266	205	29
		Inoculated with mycorrhizal fungi - Mineral Nitrogen							
P0	K0	86	120	121	93	49	68	32	5
P40	K40	376	618	559	341	314	140	190	15
P40	K160	207	490	598	386	311	324	211	32
P160	K40	1115	704	482	299	150	38	2	0
P160	K160	973	1836	1152	597	596	286	106	2

APPENDIX 14

The amount of acetylene reduced (μM/10 pl/hr) by white clover on seven successive occasions, when treated with a mycorrhizal fungus and nitrogen, phosphorus and potassium fertilizers (Experiment 9)

Harvest		1	2	3	4	5	6	7
Growth Period (days from sowing)		0-35	35-48	48-62	67-76	76-90	90-123	123-151
Length of Growth Period (days)		35	13	14	14	14	33	28
Treatment		Uninoculated - Fixed Nitrogen						
P0	K0	0	0	0	0	0	0	0
P40	K40	0	0.2	5.8	4.0	2.2	1.1	2.7
P40	K160	0	0	2.3	6.0	6.2	3.9	2.3
P160	K40	9.1	6.8	6.2	4.3	1.7	1.5	1.2
P160	K160	11.6	26.7	15.0	8.9	2.8	3.2	1.5
		Inoculated with a mycorrhizal fungus - Fixed Nitrogen						
P0	K0	0	0	0	0.4	0.4	0.1	0.5
P40	K40	0.4	3.1	4.4	3.8	2.6	1.6	2.6
P40	K160	0.2	3.6	7.9	6.1	4.6	2.5	4.4
P160	K40	10.3	7.0	8.6	3.9	2.3	1.4	1.8
P160	K160	8.6	25.6	29.4	11.4	5.4	3.4	3.8

APPENDIX 15

The number of flowers on white clover sampled on eight successive occasions, when treated with a mycorrhizal fungus and nitrogen, phosphorus and potassium fertilizers (Experiment 9)

Harvest		1	2	3	4	5	6	7	8
Growth Period (days from sowing)		0-35	35-48	48-62	62-76	76-90	90-123	123-151	151-193
Length of Growth Period (days)		35	13	14	14	14	33	28	42
					Uninoculated - Fixed Nitrogen				
P0	K0	0	0	0	0	0	0	0	0
P40	K40	0	0.2	0.8	0.4	0.2	0.2	0	0
P140	K160	0	0	0.2	0.2	0.6	1.8	0.6	0.6
P160	K40	0	0.6	3.2	0.4	0	0	0	0
P160	K160	0	3.2	8.0	1.0	1.0	0	0.2	0
					Uninoculated - Mineral Nitrogen				
P0	K0	0	0	0	0	0	0	0	0
P40	K40	0	0.2	0.4	0	0	0	0	0
P40	K160	0	0	0.2	0.4	0	0.4	0.2	0
P160	K40	0	2.2	1.2	0	0	0	0	0
P160	K160	0	7.6	10.0	2.2	1.0	0	0	0
					Inoculated with a mycorrhizal fungus - Fixed Nitrogen				
P0	K0	0	0	0	0	0	0	0	0
P40	K40	0	0.4	3.4	0.2	0	0.4	0	0
P40	K160	0	0	2.8	1.8	0.2	0.8	0.2	0.4
P160	K40	0	1.0	3.0	0.8	0	0	0.2	0.4
P160	K160	0	1.8	7.0	1.4	0.2	0	0.2	0.8
					Inoculated with a mycorrhizal fungus - Mineral Nitrogen				
P0	K0	0	0	0	0	0	0	0	0
P40	K40	0	0	0.4	0.4	0	0	0	0
P40	K160	0	0	0.2	1.2	0.4	0.2	0	0
P160	K40	0	1.4	3.4	0.2	0	0	0	0
P160	K160	0	1.6	7.0	2.2	0.2	0.2	0	0

APPENDIX 16

The dry matter yield (kg/ha) of white clover taken from Sourhope, sampled on two occasions, and treated with mycorrhizal endophytes and superphosphate (Experiment 15)

Superphosphate (kg/ha)	Inoculant	Dry Matter Yield (kg/ha)	
		4/7/79	10/10/79
0	None	376	694
	G.mosseae L1	509	762
	G.caledonius	225	373
40	None	423	418
	G.mosseae L1	464	662
	G.caledonius	822	1108